Tuesday, July 29, 2014

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

Cancer/Tumor Markers

A-001

The expression of post-translational modification of Alpha-1-antichymotrypsin in the plasma of colorectal cancer

H. Lin[1], C. Liao[2], J. Lin[3], K. Lee[3], Y. Chen[3], H. Ning[4], C. Lin[3]. *[1]Chang Gung Memorial Hospital and School of Medical Laboratory Science and Biotechnology, Taipei Medical University, Taipei, Taiwan, [2]Proteomics Research Center, National Yang-Ming University, Taipei, Taiwan, [3]School of Medical Laboratory Science and Biotechnology, Taipei Medical University, Taipei, Taiwan, [4]Chang Gung Memorial Hospital, Tao-Yuan, Taiwan*

Colorectal cancer is the third major cause of cancer related death in the world according to the report of cancer statistics in 2013. At present, some screening biomarkers are applied for the detection of colorectal cancer but most of them do not have good specificity and sensitivity. Carcinoembryonic antigen (CEA) is the most use of tumor markers for colorectal cancer, but the specificity and sensitivity is poor. In this study, we used proteomic approaches to investigate the expression of post-translational modification (PTM) of alpha-1 antichymotrypsin (ACT) in the plasma of colorectal cancer. We used immunoprecipitation, western blot and nano-LC/MS/MS to analyze the plasma samples from normal and cancer groups. Then we compared some types of PTM between those samples in order to find out the useful PTM sites in ACT to be a diagnostic tool. In the result of immunoprecipitation, we identified the accurate site of ACT in the gel electrophoresis. The following result of western blot showed that there was no significance between the normal group and the early stage of colorectal caner group (p=0.010). However, it was significant between the normal and the late stage group (p<0.001). From the result of mass analysis, we identified four types of PTM in ACT, such as Hydroxylation (Asn-323), Methylation-2 (Glu-334), 4-Hydroxy-2-nonenal (Arg-298) and N-glucuronylation (Ser-415). The expression level of Hydroxylation was increased by 2-fold in colorectal samples when compared with normal samples (p<0.05). The sensitivity of that was 88.89% and the specificity was 77.78% (AUC=0.840). Our results suggest that using the expression level of PTMs in ACT would be applicable as biomarkers for the early detection of colorectal cancer.

A-002

Tumor Marker, Molecular, and Imaging Test Monitoring of a Non-Toxic Adjuvant Integrative Nutritional Therapy Option for Stage IV Brain, Lung, Prostate, and Breast Cancer Patients When Traditional Therapy Options Have Been Exhausted: Palladium Lipoic Acid Complex, Coenzyme Q10, and Vitamin D Impacting the Mitochondrial Reactive Oxygen Species (ROS) Production and Apoptosis

E. J. Neren. *Edward J. Neren, Biomedical Consultant, Suffern, NY*

Background: Metal compounds (Platinum, etc.) have been used as cancer therapies for years; however, patient toxicity usually results. Dr. Merrill Garnett synthesizing organo-metallic compounds (1960-1990) encapsulated the metal palladium in alpha lipoic acid (non-toxic and successful in treating mice with Ehrlich carcinoma). Cat/dog tumors were also successfully treated. Rudy Falk, MD (1992 University of Toronto) determined safety, improvement, and many remissions in gravely advanced cancer cases. Since then, 200+ U.S. physicians have used the palladium/lipoic acid complex (PdLAC), Coenzyme Q10, and Vitamin D as an adjuvant integrative nutritional therapy for late stage cancer patients.

Objective: To provide stage IV cancer patients/physicians with a documented nutritional therapy option that justifies physician calls to other physicians with clinical experience, regarding their latest clinical data and determine if the therapy monitored with tumor markers, molecular, and imaging testing is appropriate for the given patient.

Methods: After determining patient levels (tumor markers, Coenzyme Q10, and Vitamin D) PdLAC liquid/coenzyme Q10/Vitamin D is physician monitored and taken as nutritionals. PDLAC is water and fat soluble and impacts the mitochondrial ROS of both cancer and normal cells. Positive clinical response and improved quality of life results is expected within three months of intake. Eight to twelve PdLAC teaspoons is taken in juice 4 times a day (based on patient body weight - 1 teaspoon for each 30 pounds). This therapy seeks balance between therapy, nutrition, detoxification, and energy enhancement. Patient progress is monitored with traditional clinical chemistries, tumor markers, Coenzyme Q10, Vitamin D testing and imaging.

Mechanism: The PdLAC enters normal and cancer cells and the mitochondrial outer membrane by the voltage dependent anion channel, then through the inner membrane by the Complex 1. The oxidative phosphorylation (OXPHOS) channel produces low levels of ATP in the cancer cell. PdLAC (acting as an electrical shunt) in normal cells would ordinarily give off electrons to the OXPHOS producing more ATP. In the cancer cell (damaged OXPHOS) it donates electrons producing increased reactive oxygen species (ROS). The excessive ROS builds up between the outer and inner mitochondrial membranes. When the outer membrane ruptures, the ROS, Cytochrome C, and the Procapases 2, 3, and 9 enter the anaerobic cytoplasm of the cancer cell and death occurs.

Results: James Forsythe, MD, HMD conducted out-come based stage IV studies (500+ patents 2004-2012) He found improvement in quality of life issues directly proportional to improvement to overall response rate and found stable disease can be tolerated and transformed into chronic livable condition.

Conclusion:This clinical and scientific documentation/data, from several public/professional sources, provides a non-toxic adjuvant integrative nutritional therapy option for advanced diseased cancer patients/physicians, when traditional therapy options have been exhausted and non-traditional therapy options are under consideration. Physician calls to other physicians with PdLAC, Coenzyme Q10, and Vitamin D clinical experience, can determine if this therapy and monitoring of patient progress is appropriate for a given end stage patient. It is not meant to circumvent physician patient monitoring, good medical practice, medical ethics, and/or negatively impact the physician's license.

A-004

Epidermal Growth Factor Receptor (EGFR) gene mutations frequency in Brazilian lung adenocarcinoma samples by pyrosequencing.

V. D. T. Niewiadonski, P. Y. Nishimura, O. Fernandes, N. Gaburo Jr.. *DASA, Sao Paulo, Brazil*

Background: Lung cancer is the most prevalent life-threatening cancer worldwide with more than 80% being non-small cell lung cancer (NSCLC). Detection of mutations of EGFR gene is critical for predicting the response to therapy with tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib, in patients with NSCLC. Patients that are EGFR mutants have constitutive TK activity and, therefore, a greater sensitivity to anti-EGFR inhibition.

Objective: To describe the EGFR mutations frequency found in lung adenocarcinoma samples, using pyrosequencing method.

Method: Thirty samples of lung adenocarcinoma were analyzed from January 2013 to December 2013. The test was performed on formalin-fixed, paraffin-embedded tumor specimen, after the selection of the specimen region to be analyzed by a pathologist. The DNA was extracted using the Qiaamp FFPE Tissue kit (Qiagen, Hiden, Germany). Concentration of DNA sample was measured spectrophotometrically using a NanoDrop spectrophotometer (NanoDropTechnologies, Wilmington). Codons 719, 768, 790, 858, 861 and exon 19 were amplified by PCR using the EGFR Pyro kit (Qiagen, Hiden, Germany). Successful and specific amplification of the region of interest was verified by visualizing the PCR product on capillary electrophoresis using Qiaxel DNA Screening Kit (Qiagen, Hiden, Germany). Preparation of single-stranded DNA was done using PyroMark Q24 vacuum workstation (Qiagen, Hiden, Germany) according to the manufacturer instructions. The pyrosequencing reaction was analyzed on the Pyro Mark Q24 (Qiagen, Hiden, Germany)

Results: The frequency of EGFR mutations found is presented on Table 1. All mutations together represent only 27% of the samples.

Conclusion: The results are consistent with previous studies and reports. The single-point mutation L858R (CTG> CGG) on exon 21 and the frame deletions on exon 19 represents the majority mutations found in Brazilian lung adenocarcinoma samples, although most samples showed no mutation at the target regions.

Table 1. Frequency of EGFR mutations found in lung adenocarcinoma samples.

Results	Frequency
Wild type	73%
2235del15 (exon 19)	3,3%
2236del15 (exon 19)	3,3%
2237_2255>T (exon 19)	3,3%
2239_2248>C (exon 19)	3,3%
CTG>CAG (L861Q)	3,3%
CTG>CGG (L858R)	10%

A-005

Ovascreen Lateral Flow Device for Simultaneous Detection of CA125 and WFDC2 (HE4) in Ovarian Cancer

Y. Lebedin. *Xemabio LLC, Gainesville, FL*

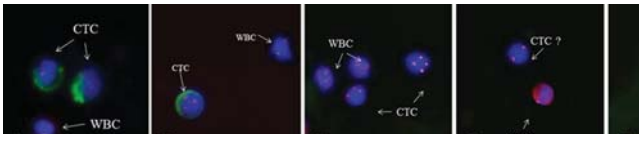
A lateral flow device named Ovascreen has been developed to simultaneously detect two tumor markers (CA125 and HE4) in ovarian cancer. Combined determination of serum HE4 (WFDC2 protein) and CA125 is demonstrated to increase the sensitivity and accuracy of early diagnosis and monitoring of ovarian carcinoma. However, a convenient and portable device for such a dual detection is not commercially available. In this study, a panel of monoclonal antibodies of HE4 (WFDC2) was obtained by immunization of human tumor-derived antigens at Xema Company. The HE4 antibodies were classified into 3 epitope groups for antibody matching based on their cross-inhibition and bindings to recombinant and natural antigens. On the other hand, the antibodies of MUC16 (antigen CA125) were also developed and the matched pairs (X306 and X325) were obtained by Xema. The selected pairs of both CA125 and HE4 were coated with colloidal gold particles, and combined onto a lateral flow device. Their assay performance was evaluated based their stability and their signal to background ratios. The best pair of CA125 and HE4 antibody-gold conjugates for lateral flow device were determined. The cut-off values for CA125 and HE4 on the device is set as 35 U/ml and 150 pmol/l, respectively. The resulting Ovascreen cassette device is validated with clinical samples of whole blood, serum, and plasma. 74 samples from primarily diagnosed but untreated serious adenocarcinoma of the ovary were evaluated. Ovascreen device showed excellent sensitivity and accuracy during the clinical evaluations. The combined positive results on Ovascreen device accounted for more than 50% cases in 69 patients which corresponding to 93% clinical sensitivity. 66 out of 69 Ovascreen positive results were further confirmed by commercial ELISA kits of CA125 (Xema Co.Ltd.) and HE4 (Fujirebio Inc.). The Ovascreen lateral flow device is suggested for use in POC based diagnosis and monitoring of ovarian cancer.

A-006

Characterizations of Circulating Tumor Cells Identified by Combination of Fluorescence in situ Hybridization and Immunostaining CK, CD45 in Pancreatic Cancer

Y. Zhang, N. Ning, Q. Chen, F. Wang, W. Cui. *Peking Union Medical College Hospital Chinese Academy of Medical Sciences, Beijing, China*

Background: To improve the identification for CTCs with weak or negative CK and diploid CTCs in pancreatic cancer, we combined the immune-staining of CK, CD45, DAPI and fluorescence *in situ* hybridization with the centromere of chromosome 8 (CEP8) probe method. **Methods**: CTCs in 3.75 mL of blood were negatively enriched with Epithelial Cell Adhesion Molecule independent magnetic beads coated with anti-CD45 antibodies and identified by combining CK, CD45, DAPI and CEP8 in 61 cases including 22 pancreatic cancers, 3 borderline pancreatic solid pseudopapillary tumors, 6 pancreatic benign tumors, and 30 healthy individuals. **Results:** Enriched cells could be classified into 5 patterns (Fig. A-E): CK+CD45-DAPI+CEP8=2 (2 hybridization signals), CK+CD45-DAPI+CEP8>2 (>2 hybridization signals), CK-CD45-DAPI+CEP8>2, CK-CD45-DAPI+CEP8=2, and CK+/-CD45+DAPI+CEP8=2or>2. Among 22 pancreatic cancers, the patterns of CK+CD45-DAPI+CEP8=2 and CK+CD45-DAPI+CEP8>2 were identified in 2 cases, the number of CTCs was 6, 12 cells/3.75mL and 2, 44 cells/3.75mL, respectively. The pattern of CK-CD45-CEP8>2 was identified in 16 cases with the range of 1-14 cells/3.75mL and the median of 3 cells/3.75mL. The pattern of CK-CD45-CEP8=2 and CK+/-CD45+CEP8=2 or >2 were detected in both pancreatic cancers and other control cases. Dynamically monitoring CTCs and platelet count prior to and after surgery in 7 pancreatic patients revealed that they were consistent both decreased or insignificantly decreased 3 days after surgery, whereas the count significantly increased 10 days after surgery. **Conclusion:** The patterns of CK+CD45-DAPI+CEP8 =2, CK+CD45-DAPI+CEP8>2 and CK-CD45-DAPI+CEP8>2 were considered as CTCs, and the patterns CK-CD45-CEP8=2 and CK+/-CD45+DAPI+CEP8=2 or >2 were considered as indeterminate cells. Postoperative increase in the platelet count might contribute to CTCs dissemination, and certain correlation might exist between those two events.



A-007

Pca3 gene expression as biomarker to differential diagnosis of benign hyperplasia and prostatic cancer

F. F. Coelho[1], O. Romano[2], C. Corradi[1], L. Nogueira[1], W. Cabral[1], E. Mateo[3], K. Borges[1]. *[1]Federal University of Minas Gerais, Belo Horizonte, Brazil, [2]Hermes Pardini Institute, Vespasiano, Brazil, [3]Hermes Pardini Institute, Belo Horizonte, Brazil*

Background: The Prostate Cancer (Pca) is the second most common type of cancer in men around the world. Because of the increasing numbers of cases, it is extremely important the development of a noninvasive test with high specificity and sensitivity to diagnosis cancer and other prostatic alterations. Studies showed that the gene 3 of Prostate Cancer (PCA3) presents high levels of expression in tumor tissue. High levels of PCA3 gene expression can be associated with an increased probability of positive biopsy and has arisen as a molecular marker in the diagnosis of PCa.

Objective: We proposed to evaluate the the expression of PCA3 gene in urine from patients with benign hyperplasia (BPH) or prostatic cancer.

Methods: The study included 33 men attended at the Clinical Hospital from Federal University of Minas Gerais (HC-UFMG) to performer a prostatic biopsy, being 13 patients with Pca, 8 with BPH and 12 patients with no alterations (controls). It was collected 30 mL of patient's urine after prostatic massage, which was immediately centrifuged. The pellet was added to RNA later® and stored for up to 24 hours, until the extraction of RNA. The samples were quantified in a spectrophotometer and submitted to treatment with DNase. After, this sample was quantified in a one-step RT-PCR for PCA3 gene and PSA/ ACTB genes for control or reaction normalization.

Results: The PCA3 gene expression was detected in 10 patients with PCa, 3 with BPH and 2 controls. For the remaining patients was not detected any gene expression. The test presented 77% of sensitivity for PCa screening and 38% for BPH. The specificity was 83% for both.

Conclusion: The PCA3 screening showed median sensitivity for PCa diagnosis; subsequently prostate biopsy is still considered the best standard procedure for detect prostatic alterations. Some patients with PCa no presented any expression of the PCA3 gene, which can be explained by the large number of interfering, as well as the prostate massage, the use of drugs and the high RNA degradation's rate in urine samples. It is required the standardization of these procedures and to analyze a larger number of samples in order to evaluate the importance of PCA3 gene expression in differential prostatic alterations and its use in the clinical practice.

A-010

Circulating tumor markers of benign and malignant disorders of breast in Libya.

J. R. Peela[1], S. Shakila[1], S. J. Dhoipode[2], A. R. Said[1], H. Beloch[2], S. Nang[2], L. T. Peela[3], A. M. Jarari[4], S. O. Alsaeoti[5], H. El Awamy[4], F. Elshaari[4], N. M. Jarari[6], M. J. Kadeer[4]. *[1]Department of Biochemistry, Faculty of Medicine,Quest International University Perak, Ipoh, Malaysia, [2]Faculty of Medicine,Quest International University Perak, Ipoh, Malaysia, [3]Great Eastern Medical School, Srikakulam, India, [4]Department of Biochemistry, Faculty of Medicine,University of Benghazi, Benghazi, Libyan Arab Jamahiriya, [5]Department of Surgery, Faculty of Medicine,University of Benghazi, Benghazi, Libyan Arab Jamahiriya, [6]Department of Pharmacology, Faculty of Medicine,University of Benghazi, Benghazi, Libyan Arab Jamahiriya*

BACKGROUND Breast cancer is most common malignant disorder in Libyan females. Breast cancer is the most dreadful disease in terms of quality of life, though heart disease is a more common cause of mortality here. The present study is a case control study of tumour markers CA 125, CA 15-3 and Carcino Embryonic Antigen (CEA) in serum of patients suffering from benign and malignant breast disorders in Libya.

Materials and methods: There are 12 cases of carcinoma of breast patients with age group ranging from 30 - 55 years of age, 10 cases of benign breast disorders i.e., Fibroadenosis with age group ranging from 18 to 50 years retrieved from department of surgery, 7th October Hospital, Benghazi, Libya and there are 12 cases of age matched controls free from both malignant and benign disorders of breast were included in this study. Venous sampling was done to the patients, all the patients and controls were measured serum CA125, CA15-3and CEA levels by authenticated methods by using Cobas E 411 analyser. Statistical analysis was done by using SPSS software by using Mann-Whitney and Wilcoxon tests.

Results: There is no significant rise of CA 125 in benign (p=0814) and malignant

(p= 0.676) disorders of breast when compared to controls. CA 15-3 was significantly high in patients suffering from breast cancer (p= 0.019) when compared to controls and also very significantly high when (p= 0.003) compared with patients with benign breast disorders. The level of CA 15-3 was not significantly high in patients with benign breast disorders (p=0.186) when compared to controls. The level of CEA was significantly high in patients of breast cancer when compared to patients of benign breast (p=0.009) disorders and (p=0.017) controls. The level of CEA is not significantly in high patients of benign breast disorders (p=0.634) when compared to controls.

Conclusion: The present study showing high levels CA 15-3 and CEA only in malignant disorders of breast may be useful as diagnostic and prognostic markers. CA 125 has not shown any significance in this study proving that it is not an important marker in malignant breast disorders.

A-011

Diagnostic value of Insulin-like growth factor-1, IGF-binding protein-3, Chromogranin-A in differentiation between benign prostatic hyperplasia and prostate cancer patients

S. A. K. Saleh. *Umm AlQura University, Makkah, Saudi Arabia*

Backgrounds: Prostate cancer (PCa) ranked the sixth most common cancer among males in Arab world. Elevated serum Insulin-like growth factor-1 (IGF-1) level appeared to be a possible risk factor for the development of PCa. Chromogranin A (CgA) is the most employed serum marker to detect neuroendocrine features. Many studies reported ***contradictory*** findings of association between IGF-binding protein-3 (IGFBP-3) and the risk of PCa. 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) in patients with moderately increased PSA levels. **Objective:** This study aimed to explore the validity of IGF-1, IGFBP-3, CgA and thereof ratios with PSA in differentiation between BPH and PCa patients in Saudi Arabia. **Patients and Methods:** The study included 62 patients with PCa, 70 BPH patients and 56 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 of the prostate, computed tomography (CT) scan of the pelvis, bone scan and histopathological examination, accordingly PCa stages and metastatic disease were confirmed. PCa patients were classified into localized (n = 48) and metastatic PCa (n = 14). Serum levels of IGF-1, IGFBP-3, CgA, 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 87% at 85% specificity with an accuracy of 86%. Although serum IGF-1, IGFBP-3 and CgA levels did not differentiate among PCa, BPH and control groups ($p>0.05$), IGF-1/PSA as well as IGFP-3/PSA ratios were found to differentiate significantly among metastatic, localized PCa, BPH and control groups ($p<0.05$). Combined use of IGF-1/PSA and IGFP-3/PSA ratios provide an overall value of sensitivity, specificity and diagnostic accuracy (92, 84 and 88% respectively) in the diagnosis of PCa. The addition of f/t PSA ratio to this combination seems to improve the overall value of sensitivity, specificity and diagnostic accuracy (94, 85 and 90% respectively). **Conclusion:** Although there is no association of PCa risk with serum IGF-I and IGFBP-3 levels; combination of these growth factors with PSA and f/tPSA may be useful and can improve the overall value of sensitivity, specificity and diagnostic accuracy of patients with PCa. Further studies are needed to elucidate the prognostic and predictive value of these growth factors as well as their association with PCa risk.

A-012

Stability of Serum Human Epididymis Protein 4 (HE4)

R. Radwan[1], C. Fermer[2], M. Lundin[2], S. Raju[1], S. Jones[1], C. Hall[2], D. Dickson[1], T. Kettlety[1], Z. Li[1]. *[1]Fujirebio Diagnostics Inc., Malvern, PA, [2]Fujirebio Diagnostics AB, Gothenburg, Sweden*

Background: HE4 is encoded as a 13 kDa protein and belongs to the family of whey acidic four-disulfide core (WFDC) proteins (Israeli O et al, 2005; Bouchard D et al, 2006; Bingle L et al, 2002). HE4 was first identified in the epithelium of epididymis (Kirchhoff C et al, 1991; 1998). Secreted HE4 has become an important biomarker for the detection of ovarian cancer, a common cause of cancer-related death in women, with 67% sensitivity and 96% specificity (Hellstrom I et al, 2003). Furthermore, the combination of HE4 and CA 125 has been demonstrated to be a more accurate predictor of ovarian cancer with a sensitivity of 76% and a specificity of 95% (Moore RM et al, 2008). The purpose of this study was to determine the stability of the serum analyte HE4 under frozen conditions.

Methods: This is a retrospective study utilizing banked serum samples collected in an ovarian cancer clinical trial (NCT00315692). The patients were from women greater than or equal to 18 years of age, who selected to undergo laparotomy or laparoscopy based on finding of a pelvic mass. The samples were collected in the US under an IRB approved protocol. Samples were collected in red top tubes or serum separator tubes (SST); and undergone no more than five (5) Freeze/Thaw cycles prior to the date for stability testing. Samples were stored in a ≤-70°C freezer since the time of collection. Sample testing was performed using the manual HE4 EIA, a sandwich immunoassay. The initial HE4 EIA testing was carried out in July to August of 2007 and the results were retrieved from the clinical trial dataset. The stability testing with the HE4 EIA was carried out in June of 2013. A total of 100 available samples from this trial were tested. Among them, 84 from women with benign disease, 11 from women with border line/low malignant potential, 4 from women with ovarian cancer, and 1 from a woman with other gynecological cancer.

Results: The linear correlation coefficient between the two measurements was 0.986 (95% CI: 0.979 to 0.991). Weighted Deming regression gave an intercept of 2.76 (95% CI: -0.54 to 6.07); and a slope of 1.033 (95% CI: 0.970 to 1.095). The intercept and slope are not significantly different from 0 and 1 respectively. Passing-Bablok regression produced a similar intercept of 3.06 (95% CI: -1.26 to 6.35) and slope of 1.031 (95% CI: 0.965 to 1.108).

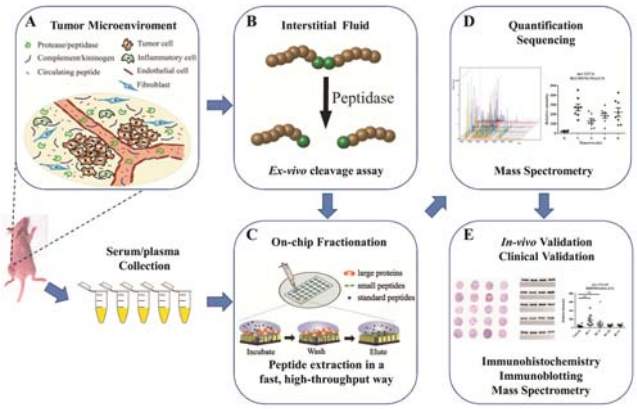
Conclusion: The human serum HE4 was demonstrated to be stable for at least five (5) years for the serum samples stored at ≤ -70°C and underwent no more than five (5) Freeze/Thaw cycles.

A-013

Circulating proteolytic products of tumor-resident enzyme as potential biomarkers for early detection of breast cancer

T. Hu. *Houston Methodist Research Institute, Houston, TX*

In theory, any or all of tumor-secreted proteins can serve as cancer biomarkers. However, the reality is challenging to monitor because of the large degree of fluctuation in abundance and localization of these tumor-secreted proteins, especially in the early stage of tumor development and/or metastasis. As such, it seems feasible that we might take advantage of the fact that secreted proteases/peptidases in the tumor microenvironment generate proteolytic products, also referred to as "circulating peptides", which are detectable in bloodstream and provide ample information about the body, "coded" in the patterns and quantity of these peptides. Herein we clearly link the catalytic activity of Carboxypeptidase N (CPN) to its proteolytic products during breast tumor progression in mouse model and clinical samples. CPN plays important roles in regulating vasoactive peptide hormones, growth factors, and cytokines by specifically cleaving their C-terminal basic residues. Circulating fragment profiling, by an approach combining nanopore fractionation and mass spectrometry, revealed the nature and extent of cleavage by CPN. These results correlated with the level of CPN-catalyzed peptides in blood taken from the tumor-bearing mice, healthy women and breast cancer patients. We showed that generation of C3f_R1310-L1319 specifically correlated with the CPN expression level. In both mouse and clinical patient samples, the amount of CPN was increased in tumor tissues compared to that seen in normal breast tissue, while its counterpart in blood remained constant. The amount of 6 CPN-catalyzed peptides predominantly increased in sera taken from both the mice at 2 weeks after orthotropic implantation and the patients' plasma as early as the first pathologic stage of breast cancer. In conclusion, the circulating level of the selected 6 CPN-catalyzed proteolytic products reflects the extent of this enzyme's activity in tumors, and our results clearly indicate their strong potential as biomarkers for non-invasive early diagnosis of breast cancer.



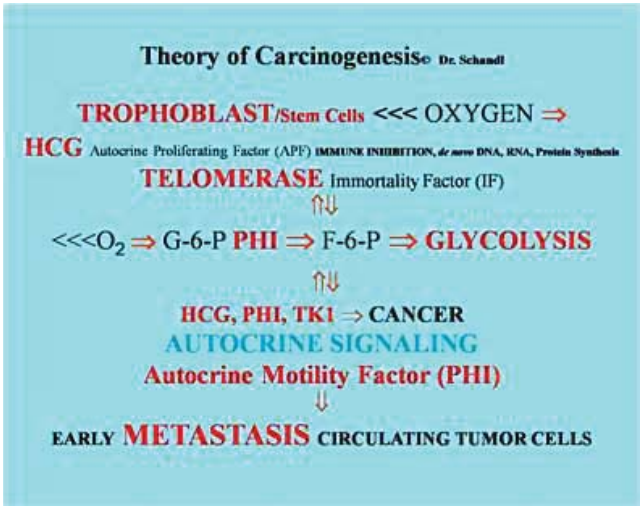
A-016

Clinical Utilization of the Cancer Profile and Longevity Profile and the Role of Thymidine Kinase (TK1) in Carcinogenesis

E. K. Schandl. *American Metabolic Laboratories, Hollywood, FL*

Cancer is usually detected by visualization. Clinical biochemical parameters, e.g. Cancer Profile (CA Profile) and Longevity Profile (expanded CA Profile), are capable to signal a developing malignancy much earlier. The methodologies used in this study are: Chemiluminescence (HCG, CEA, TSH, DHEA-S), IRMA (HCG) and enzyme kinetics (PHI, GGTP). *HCG*, the pregnancy/malignancy hormone is the *autocrine proliferative factor* (APF). It is responsible for *de novo* DNA, RNA, protein synthesis in pregnancy and may as well be so in malignancies. *PHI* is a neurokine. Amongst its many other functions it is the *autocrine motility factor* (AMF). PHI regulates anaerobic sugar metabolism by facilitating the Warburg effect. This enzyme is responsible for cytokines and as such, may be the facilitator of micrometastesis and circulating tumor cells by attaching to their membrane receptor (neurokine) and "jockeying" cells to a distant site. Thymidine Kinase (*TK1*) is a dynamic measure of tumor growth. It phophorilates deoxythymidine to deoxythymidine monophosphate, a prerequisite for DNA replication. All three factors, HCG, PHI, TK1, must be present for the development and sustenance of malignancies. Our data confirms the presence of PHI and HCG in most, if not all cancers. TK1 was found in cancer biopsies, but not in normal tissues. Reports showed the presence of the enzyme in embryonic, wound healing, and tumor cells.

Clinical laboratory results confirmed positive CA Profile markers in approximately 90% of hundreds of pathologically established malignancies. Breast cancer yielded 92% positives, lung 97%, and colon 93%. The clinical laboratory adaptation of the proposed profile may warn of developing, undiagnosed cases, and track progress monitoring. The Longevity Profile is a conglomerate of laboratory tests for cancer, coronary risk factors, sex hormones, bone health, adrenal stress, and generally an overall examination of most organs. It is a biochemical full body scan without radiation.



A-017

WHAT ARE THE TUMOURS MARKETS THAT BEST IDENTIFY MALIGNANT PLEURAL EFFUSIONS?

J. D. Santotoribio, A. García de la Torre, C. Cañavate-Solano, F. Arce-Matute, S. Pérez-Ramos. *Puerto Real University Hospital, Cádiz, Spain*

Introduction Malignant pleural effusions (MPE) are a common clinical problem in patients with neoplastic disease. 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) measurement in pleural fluid for diagnosis of MPE.

Materials and methods We studied pleural fluids obtained by thoracocentesis in patients with pleural effusion (PE). We measured CEA, CA 15.3, CA 19.9 and CA 125 in pleural fluid by electrochemiluminescence immunoassay in MODULAR E-170 (ROCHE DIAGNOSTIC®). Patients were classified into two groups according to the aetiology of PE: benign PE (BPE) and MPE. PE was categorized as MPE if malignant cells were demonstrated in pleural fluid or pleural biopsy. The accuracy for diagnosis of MPE was determined using receiver operating characteristic (ROC) techniques by analysing the area under the ROC curve (AUC).

Results We studied 152 patients with ages between 1 and 89 years old (median = 61.5 years old). Fifty-one patients were MPE (22 lung cancer, 9 breast cancer, 7 mesotheliomas, 5 lymphomas, 4 kidney cancer, 2 colon cancer and 2 ovarian cancer) and 101 were BPE (46 transudates, 42 parapneumonic, 5 tuberculous, 5 pulmonary thromboembolism, 2 quilotórax and 1 rheumatoid arthritis). No statistically significant differences were found between MPE and BPE patients according to CA 125 ($p>0.05$). AUC values was 0.815 ($p=0.0024$) for CA 15.3, 0.682 ($p=0,0064$) for CEA and 0.639 ($p=0,0359$) for CA 19.9. Optimal cut off value were 16.2 U/mL (61.5% sensitivity and 86.3% specificity), 2.3 ng/mL (57.7% sensitivity and 76.6% specificity) and 2.4 U/mL (64.3 sensitivity and 62.3% specificity) for CA 15.3, CEA and CA 19.9 respectively.

Conclusions CA 15.3 levels improved accuracy for diagnosis of MPE compared with CEA, CA 19.9 or CA 125. CA 15.3 showed high diagnosis efficacy to predict whether a pleural effusion is benign or malignant.

A-018

Relation of total antioxidant capacity and CA 125 in patients with epithelial ovarian carcinoma

L. Kurti[1], I. Osmani[1], A. Kelmendi[1], L. Zeneli[2]. *[1]University of Prishtina, Faculty of Medicine, Institute of Biochemistry, Prishtina, Kosovo, Republic of, [2]University Clinical Centre of Kosova, Institute of Clinical Biochemistry, Prishtina, Kosovo, Republic of*

Background: We undertook the present study to investigate the possible relation between total antioxidant capacity (TAC) and CA 125 values in serum samples of patients with epithelial ovarian carcinoma.

Methods: Serum total antioxidant capacity was measured using trolox equivalent antioxidant capacity (TEAC) assay in 20 serum samples with elevated values of CA 125 and 20 age- and sex-matched healthy subjects (controls) with CA 125 values within reference ranges respectively.

Results: The measured level TAC was higher in serum samples from patients with elevated CA 125. TAC correllated significantly with CA 125 ($r: 0.516$, $P<0.05$) when the values of this tumor marker were pathological.

Conclusion: This study suggests that increased serum TAC of the patients with altered levels of CA 125 may be due to the response of increased reactive oxygen species and can be considered as a sign of oxidative stress of these patients. Therefore, the evaluation of serum antioxidant capacity in patients with epithelial ovarian carcinoma could contribute in diagnosis of these patients.

A-020

Experience with the use of the CellSearch system for enumeration of circulating tumor cells (CTCs) in Asian subjects

T. C. Aw, M. Goh, S. Swe. *Singapore Institute of Advanced Medicine, Singapore, Singapore*

Background: Circulating tumor cells (CTCs) are increasingly used as independent prognostic markers as well as predictors of response to anti-tumor treatment. Solid tumors are derived from the epithelium. Metastasis is initiated when tumor cells invade the blood stream. Normally absent in blood, these circulating epithelial-

derived cells can be captured using antibodies directed against the epithelial cell adhesion molecule (EpCAM). The CellSearch CTC system (Janssen Diagnostics) is the only FDA approved platform for CTC detection of metastatic cancers from the breast (since 2004), colon (2007), and prostate (2008). We acquired the CellSearch system recently and now describe our experience with its use in providing a clinical service for CTC analysis in the Asia-Pacific region.

Methods: The CellSearch system comprises an automated CellTracks AutoPrep instrument to capture and label the CTCs and a semi-automated immuno-fluorescent microscope (CellTracks Analyzer) for cell detection. Blood, collected in proprietary tubes, are stable for up to 96 hours prior to analysis. Buffer containing ferrofluid (nanoparticles with a magnetic core and an outer layer coated with anti-EpCAM antibodies) is added to the sample. Following immuno-magnetic capture and enrichment, CTCs are permeabilized before exposure to fluorescent antibodies directed against the cytoplasm (anti-CK-8,18,19), nucleus (DAPI) and leucocytes (anti-CD45). Thereafter the mixture is transferred into a plastic cartridge surrounded by a magnetic sheath. The CTCs are attracted to the surface of the cartridge by the magnet. The analyzer can accommodate up to 8 samples in each run of 2-3 hours. Each assay has a control sample comprising SK-BR3 breast cancer cell lines tagged with 2 different fluorescent labels one each for a population of low CTC count (approximately 40-50 cells) and another with high CTC count (approximately 1000 cells). Following a 30 min incubation, the samples are transferred singly to the Analyzer which scans the surface of the cartridge. Fluorescent objects are imaged and displayed in a gallery for classification. CTCs are defined as CK+,DAPI+,CD45- fluorescent objects of at least 4 microns and co-location of the cytoplasmic and nuclear images.

Results: Presumably healthy men (n=45: 27-85 years, mean age 55) had less than 3 CTCs (84% < 2 CTCs); on repeat testing CTCs declined to < 2. Healthy women (n=56: 25-79 years, mean age 48) had less than 2 CTCs (98%). We have served 102 patients with metastatic cancer (Breast: n=43, age 34-71; Prostate: n=29,age 50-79; Colon: n=30, age 28-80). We have also analyzed samples from other cancers (brain, nose, tongue, lung, stomach, kidney, ovary, endometrium, cervix). Inter-assay precision using the kit controls range from 10-27% for the low control and 3-13% for the high control (n=11 control lots). External quality assurance (EQA) comprises a gallery of 50 images sent out quarterly by the manufacturer. All users have attained a score of > 90%. We have become aware of a third party EQA program which we intend to subscribe.

Conclusion: CTC testing is gaining in utility. As oncologists gain more experience with using CTC, testing will become mainstream and impact the routine clinical laboratory.

A-023

New rapid urine test for the identification and quantitation of immunoglobulin free light chains (Bence Jones Proteins)

J. Campbell, J. Heaney, P. Patel, M. Goodall, M. Drayson. *University of Birmingham, Birmingham, United Kingdom*

Background. Monoclonal κ and λ immunoglobulin free light chains (FLC) in the urine are important biomarkers for the diagnosis and monitoring of a number of plasma cell dyscrasias including multiple myeloma. To date, laboratory FLC tests provide the only means of quantitating FLC and often have a slow turnaround time that prevents early diagnosis or prompt identification of changes in disease activity. Furthermore, the gold standards for identifying (immunofixation electrophoresis; IFE) and quantitating (densitometry) FLC in the urine have a number of limitations. IFE lacks analytical sensitivity (LOD >10-20 mg/L) and interpretation is often subjective. Densitometry has high inter-test variability that contributes to an inter-lab CV% of 50-95% in the UK National External Quality Assessment Service (NEQAS), and is poorly sensitive meaning that urines need to be concentrated before measurement, sometimes up to 150-fold. Further, clinical manifestations such as proteinuria may obscure monoclonal FLC bands and makes identification and quantitation of monoclonal protein bands inaccurate. Therefore, we have developed a rapid test (Seralite®) that identifies abnormal FLC levels in unconcentrated urine or blood in 10 minutes. Seralite® quantitates κ and λ FLC levels simultaneously using highly specific anti-κ and anti-λ FLC monoclonal antibodies. **Methods:** Seralite® validation was conducted by retrospective analysis of urine from patients presenting with multiple myeloma (n=100). All samples were also measured for FLC by electrophoresis immunofixation; densitometry on concentrated urines; and a recently validated new Luminex assay that incorporates the same mAbs as Seralite®. **Results:** Seralite® displayed excellent clinical concordance with Luminex. Analysis of IFE results revealed that Seralite® had no false negatives, and correlated excellently with densitometry. **Conclusion:** Seralite® detected all FLC in urine from 100 myeloma patients at diagnosis. Prospective use of Seralite® to diagnose and monitor plasma cell dyscrasias including multiple myeloma should now be investigated. The utility of Seralite® in the context of other FLC related disorders including AL amyloidosis should also be established.

A-024

Method Comparison to Quantify Free Light Chain Changes during Serial Monitoring of Multiple Myeloma Patients

O. Berlanga, L. Adie, H. Carr-Smith, S. Harding. *The Binding Site, Birmingham, United Kingdom*

Background: Serum free light chain (FLC) measurements utilising Freelite® immunoassays are an integral part of the international myeloma working group guidelines. The assays are available on both turbidimetric and nephelometric instruments. Here we compare responses in FLC measurements between instruments in serial samples from multiple myeloma (MM) patients.

Methods: Sequential sera from 6 MM patients (5 IgGκ, 1 IgGλ; total sample number=45; median (range) sample number: 8 (5-11); mean (range) follow-up: 289 (97-525) days)) treated with bortezomib were analysed retrospectively with Freelite (The Binding Site Group Ltd, UK) on a SPA_{PLUS}®, Cobas Integra 400® and Modular P® turbidimeters and the BNII® nephelometer. Percentage change in dFLC (involved-uninvolved FLC) relative to baseline was calculated for each instrument, and correlation and agreement assessed using Pearson's linear regression, the coefficient of determination (R^2) and Bland-Altman test. Responses were assigned for each sequential sample following IMWG criteria. Agreement between instruments for response assignment was assessed using weighted kappa analysis; values >0.81 indicate near perfect agreement.

Results: Median FLCκ, FLCλ and dFLC concentrations were not significantly different between instruments (Mann-Whitney test), except for slightly higher FLCλ measurements on the Modular P. Regression analysis between analysers displayed dFLC % change slopes between 0.95 and 1.01 and intercepts between -0.06 and 0.03, with $R^2 \geq 0.96$ for all comparisons (Table 1). Bland-Altman test revealed a bias between 0.2 and 2.7% between instruments, with 95% limits of agreement no greater than ±16%. Weighted kappa analysis for response assignment was ≥0.89 for all instrument comparisons (Table 1).

Conclusion: There is good correlation and agreement for FLC changes and response assignment by Freelite on different analysers, indicating that routine monitoring of the disease would not be affected by instrument selection. Small between instrument differences in the measured FLC levels suggest not changing analysers during monitoring patients is preferable.

Table 1. Performance of Freelite immunoassays for monitoring dFLC changes on different instruments

		Correlations and agreement for dFLC % change			
		SPA_{PLUS}	BNII	Integra	Modular P
Weighted kappa for response assignment	SPA_{PLUS}	-	y = 0.96x - 0.05 R^2 = 0.97 1.7% (-14.7 to 11.4)	y = 0.99x - 0.004 R^2 = 0.98 1.1% (-10.1 to 12.3)	y = 0.95x - 0.05 R^2 = 0.98 1.6% (-12.0 to 8.8)
	BNII	0.96	-	y = 1.01x + 0.03 R^2 = 0.96 2.7% (-11.0 to 16)	y = 0.97x - 0.02 R^2 = 0.97 0.2% (-11.5 to 11.9)
	Integra	0.89	0.93	-	y = 0.95x - 0.06 R^2 = 0.97 2.5% (-14.5 to 9.4)
	Modular P	0.92	0.96	0.91	-

Red cells: Pearson's equation with slope and intercept, coefficient of determination (R^2) and % bias (95% limits of agreement) by Bland-Altman test.
Blue cells: weighted kappa with quadratic weighting; values >0.81 indicate almost perfect agreement.

A-025

Differential diagnosis the property of ascites by a novel logistic regression model

C. Lin, W. Feng, L. Leilei, S. Yuanyuan, W. Yueping, S. Jianguo. *Hospital, Nantong, China*

Background: Differential diagnosis of malignant from benign ascites has a great clinical significance for the treatment and prognosis of the disease. However, complete discrimination between malignant ascites and nonmalignant ascites has not yet been substantially improved in recent years. Herein, we established a logistic regression model on the basis of multiple ascitic indices in differential diagnosis both benign and malignant ascites. Moreover, the further assessment of its diagnostic value is presented.

Methods: A total of 103 consecutive ascitic patients were enrolled in this study. Nine biomarkers including telomerase, DNA ploidy, adenosine deaminase (ADA), lactate dehydrogenase (LDH), CEA, CA125, CA19-9, Golgi Protein 73 (GP73) and serum-ascites albumin gradient (SAAG) were measured. The data were further analyzed

by using receiver operating characteristic (ROC) curve, univariate and multivariate logistic regression to evaluate the value of differential diagnosis the property of ascites.

Results: The median values of the ascitic telomerase, LDH, CEA, CA125, CA19-9, GP73 in the malignant ascites group were 0.314, 235 U/L, 20.64 ng/mL, 306 U/mL, 45.21 U/mL and 185.1 μg/L, as they were compared with those of benign group (0.046, 109 U/L, 4.84 ng/mL, 62.13 U/mL, 19.5 U/mL, 69.8 μg/L), respectively, P<0.001. However, the concentration of SAAG in the malignant ascites group were obviously lower than that of benign ascites group (median, 7.09 g/L *vs* 19.20 g/L), P<0.001, Moreover, there was no significant difference in the concentration of ADA between the two groups (median, 8.5 U/L *vs* 8.0 U/L), P>0.05. In addition, DNA aneuploidy rate in the malignant group was significantly higher than that in the benign group (76.0% *vs* 9.4%), P<0.001. By using ROC curve, univariate and multivariate logistic regression analysis, ascitic telomerase (X_1), CEA (X_2), GP73 (X_3), SAAG (X_4) were rolled into logistic regression model:$P=1/[1+e^{(-6.320+2.351X1+2.338X2+4.246X3+3.459X4)}]$, (P: probability predictive value, e: natural logarithm). The area under the curve of the P value of the predictive probability was 0.986, the cut-off point was 0.469, the sensitivity was 96% and the specificity was 98.1%. When P≥0.469, it was predictively diagnosed as malignant ascites; vice versa, when P<0.469, it was predictively diagnosed as benign ascites.

Conclusion: Our study highlights that the novel logistic regression model is an attractive strategy in differentiating property of the ascites and justifies its value in the studies of diagnosis and therapy in malignant ascites patients.

A-026

Performance Evaluation of the Free PSA Immunoassay on the LUMIPULSE™ G1200 System

Y. Ishii, H. Murakami, H. Karasawa, C. Okamura. *Fujirebio Inc, Hachioji-shi, Tokyo, Japan*

Background: PSA, a glycoprotein with a molecular weight of 30 kDa localized in prostate glandular epithelial cells, is known to be released into blood when prostate epithelial cells are damaged by malignancies. It has been demonstrated that the measurement of PSA in blood is useful in the diagnosis of prostate cancer, follow-up, and evaluation of therapeutic effect as well as in screening for early detection of prostate cancer. Percentage of free PSA has been shown to improve diagnostic sensitivity and specificity around gray zone (4 to 10 ng/mL total PSA). Total PSA immunoassay is already available on LUMIPULSE G1200 system[1]. A method has been developed to measure free PSA and result of performance evaluation is presented.

Methods: Lumipulse G Free PSA is a chemiluminescent enzyme immunoassay (CLEIA) that uses a two-step method for analysis. In the first step, anti-free PSA monoclonal antibody coated magnetic particles are incubated with a patient sample. Following a wash, the alkaline phosphatase-conjugated anti PSA mAb are added to the mixture and incubated in the second step. Following another wash, the instrument adds substrate solution to initiate chemiluminescence reactions. The resulting reaction signals are proportional to the amount of free PSA in the sample and allow a quantitative determination of free PSA in serum and plasma.

Results: The imprecision of the free PSA assay measured over 20 days using two controls and three panels (ranging from 0.5 to 23 ng/mL) was total imprecision of < 3.3%.The calibration range of the Lumipulse G Free PSA was 0 - 30 ng/mL and showed a linear dose-response relationship within the calibration range. The Lumipulse G Free PSA correlated linearly with ARCHITECT Free PSA (Slope = 0.96; r = 0.99), Access Hybritech free PSA (Slope = 1.04; r = 0.99) and ELECSYS free PSA (Slope = 1.02; r = 0.99) within the range of 0.058 to 27.86 ng/mL via testing 120 serum samples. No hook effect was observed at 3,000 ng/mL, and no cross-reactivity was observed to PAP (1000 ng/mL). No interference was observed with unconjugated (18.3 mg/dL) or conjugated bilirubin (20.6 mg/dL), hemoglobin (487 mg/dL), triglycerides (2000 mg/dL), RF (rheumatoid factor, 1000 IU/mL) and HAMA (1000 ng/mL). The LOB, LOD, and LOQ were 0.001, 0.002 and 0.009 ng/mL with LUMIPULSE G1200, respectively. Correlation between serum tube and plasma tube (EDTA-2K, Na heparin) was tested (Slope = 0.99). Serum specimens (60 prostate cancer, 97 non cancer) whose PSA measurement values were in the gray zone (4-10 ng/mL) were tested using the Lumipulse G Free PSA and the mean percentage of free PSA (prostate cancer; 14.8%) showed significantly lower than that of non cancer (20.2%).

Conclusion: The Lumipulse G Free PSA assay appears to be an accurate and precise assay for the automated measurement of free PSA in human serum and plasma.

[1]Not available in the US

A-027

QUANTITATIVE DETERMINATION OF FREE LIGHT CHAINS (FLC) - KAPPA AND LAMBDA IN GROUP OF PATIENTS WITH ABNORMAL SERUM ELECTROPHORESIS PATTERN.

Y. S. Medeiros[1], R. Freitas[2], M. Debiasi[3]. *[1]UFSC, Florianopolis SC, Brazil, [2]Santa Luzia Laboratório Médico, Florianopolis SC, Brazil, [3]Santa Luzia Laboratório Medico, Florianopolis SC, Brazil*

OBJECTIVE : The dosage of free light chains (kappa and lambda - FLC) have been incorporated into guidelines for some hematological malignant diseases such as multiple myeloma (MM) and other monoclonal gammopathies, in order to aid diagnosis and monitoring. In physiological and pathological conditions such chains are likely to be measured in the bloodstream along with the other intact molecules. This study intends to investigate the concentration of FLC in a group of patients when compared with the reference values.

PATIENTS AND METHODS: 56 samples of patients with ages within 19 and 88 years old, which 22 were men and 34 were women were evaluated. Serum electrophoresis was performed on CAPILLARYS 2 (Sebia ®) and FLC dosage was performed by nephelometry on BN - ProSpec (Siemens®) using Siemens N Latex FLC kappa and N Latex FLC lambda. From these patients, based on renal function verification by MDRD (Modification of Diet in renal Disease Study- NKDEP) and serum protein electrophoresis results, 12 samples were excluded due to indication of potential renal dysfunction; whereas 17 with a record of monoclonal gammopathy and 27 with polyclonal gammopathy result were included.

RESULTS AND CONCLUSION: Only the results of FLC in patients with normal renal function (n = 44) were analyzed. For patients with monoclonal gammopathy (n = 17), eight presented kappa/ lambda index with approximately six times greater than the reference value, nine with normal values. Regarding patients with polyclonal gammopathy (n = 27), about 50 % of FLC results were high. Based on these data, we conclude that the inclusion of FLC assays will contribute to the clinical evaluation of monoclonal gammopathy. Regarding to polyclonal gammopathy, overproduction of free light chains as described in infectious processes, and autoimmune liver diseases, among others, the clinical value of this dosage is still under study.

A-028

Best practices in incidental clinical findings associated with Multiple Myeloma in patients attending the Emergency Service

J. L. García de Veas Silva[1], R. Rios Tamayo[2], C. Bermudo Guitarte[1], P. Navarro Alvarez[2], V. Sánchez Margalet[1], C. González Rodriguez[1]. *[1]Hospital Universitario Virgen Macarena, Sevilla, Spain, [2]Hospital Universitario Virgen de las Nieves, Granada, Spain*

Background: The presence of incidental clinical findings (bone pain, pathologic fractures, anemia, hyperproteinemia, hypercalcemia, acute kidney injury) related to Multiple Myeloma (MM) in Emergency Service and Primary Care should be studied for screening the existence of a possible MM. A quick panel based on serum protein electrophoresis (SPE) and quantification of serum free light chains (sFLC) enables sensitive quantification of monoclonal component in the study of MM. The application of this screening panel in patients with these incidental clinical finding without other diagnosis can help us to efficiently detect a possible MM in much sorter times.

Methods: we studied 5 patients where we found incidental clinical finding characteristic of MM (anemia, hyperproteinemia, intense bone pain). Sera of the five patients were sent to the Immunology Lab for the screening of a monoclonal protein. SPE were performed on CAPILLARYS 2 (Sebia) and the sFLC were measured with Freelite (The Binding Site) turbidimetric assay. Positive results of the screening panel, remit the patient to the Hematology Service to complete the study

Results: The results are shown in the table.

Conclusions: In the context of clinical symptoms (bone pain, pathologic fractures, anemia, hyperproteinemia, hypercalcemia) that alerts to a possible MM case in patients without obvious clinical diagnosis, we found the application of this protocol (SPE+sFLC) to be efficient and advisable. The combination of SPE and sFLC yields a fast and highly sensitivity approach in the screening of monoclonal gammopathies which in the context of the emergency service is of particular importance.

Case	Sex	Age (years)	Cause of Emergency	Clinical Finding at Emergency Service	SPE	sFLC	Diagnosis
1	Female	67	Severe abdominal pain	Hyperproteinemia (12 g/dl)	Large peak (4.18 g/dl)	KL= 10.47 mg/l LL=99.59 mg/l Ratio=0.11	Multiple Myeloma IgG Lambda Stage 2 ISS
2	Female	65	Infection and bone pain	Hyperproteinemia, hyperviscosity and thrombocytopenia	Large peak (3.28 g/dl)	KL=617 mg/l LL=11.1 mg/l Ratio=55.59	Multiple Myeloma IgG Kappa Stage 2 ISS
3	Female	64	Intense lower back pain	Intense back pain	Large peak (3.22 g/dl)	KL=3.15 mg/l LL=102 mg/l Ratio=0.031	Multiple Myeloma IgA Lambda Stage 3 ISS
4	Female	55	Lower back pain and she had a fall	Pathological fracture at D12	Two weak peaks (0.15 g/dl)	KL=28600 mg/l LL= 5.36 mg/l Ratio=5335.82	Light Chain Kappa Multiple Myeloma Stage 3 ISS
5	Male	12	Lower back pain and he had a fall in the school	Hypercalcemia (16.6 mg/dl)	Very large peak (4.34 g/dl)	KL=219 mg/l LL=1.01 mg/l Ratio=216.83	Multiple Myeloma IgA Kappa Stage 3 ISS

A-029

Performance Evaluation of Tumor Marker CA15-3 on Roche Cobas e601 Immunoassay Analyzer

S. St. Romain, I. Bermudez, B. Handy, E. Wagar, Q. H. Meng. *University of Texas MD Anderson Cancer Center, Houston, TX*

Backgrounds: Breast cancer is the most prevalent form of cancer diagnosed in women and is the leading cause of cancer death in women worldwide. Cancer antigen 15-3 (CA15-3) and CA27.29 are different epitopes on the same protein antigen product of the breast cancer-associated MUC1 gene. Substantial evidence has shown that elevated cancer antigen 15-3 (CA15-3) levels are associated with advanced breast cancer and metastasis. Thus, serum CA15-3 is used to monitor the therapeutic response and recurrence of breast cancer. This study was to assess the analytical performance of CA15-3 on Roche Cobas e601.

Methods: The Roche CA15-3 method is a sandwich electrochemiluminescence immunoassay that employs a biotinylated monoclonal CA15-3-specific antibody and a monoclonal CA15-3-specific antibody labeled with a ruthenium complex, forming a sandwich complex. The evaluation was performed following CLSI guidelines. The performance was evaluated for precision, lower limit of detection, linearity, and accuracy. The within-run and between-run precisions were assessed by analyzing QC material at low and high level of concentrations. The correlation between CA15-3 results on Roche Cobas e601 and CA27.29 results on Siemens Centaur was assessed.

Results: The within-run CVs for CA15-3 were 1.6% and 1.4% at the levels of 22 U/ml and 102 U/ml, respectively. The between-run CVs at low and high levels were 2.64% and 2.59%, respectively. The measuring range was determined to be linear between 1.00 - 300 U/ml. The lower limit of detection was 0.1 U/ml using measurable value obtained from zero standard + 2SD (n=20). Comparison of CA15-3 on Roche Cobas e601 with CA27.29 on Siemens Centaur showed that the slope was 1.0 (95% CI = 0.912 to 1.087) with intercept of -12.61 and correlation coefficient r = 0.967 (Deming). The mean bias was -12.65.

Conclusion: Our data demonstrates that the CA15-3 assay on Roche Cobas e601 analyzer has an excellent precision of performance with good linearity. There is a good correlation between serum CA15-3 and CA27.29. Serum CA15-3 can be precisely and accurately measured on Roche Cobas e601 in monitoring response to therapy and recurrence in breast cancer patients.

A-030

Urinary Free Light Chains in Patients With Polyclonal Hypergammaglobulinemia And/Or Renal Impairment

V. Brunel[1], B. Legallicier[2], J. Wils[3], M. Quillard[1], M. Godin[2], S. Claeyssens[3]. *[1]Medical Biochemistry, University Hospital, Rouen, France, [2]Nephrology, University Hospital - Inserm UMR1073 - IRIB, Rouen, France, [3]Medical Biochemistry, University Hospital - Inserm UMR1073 - IRIB, Rouen, France*

Background: In plasma cell dyscrasias, monoclonal free light chains (FLC) are involved in the pathogenesis of renal failure, a major cause of morbi-mortality. Besides, urinary excretions of polyclonal FLC are known to increase in patients with RI and, according to rare data, in those with hypergammaglobulinemia (H). However, evidence for polyclonal FLC-mediated injury is limited. In this study, we assessed the effect of H and/or RI on urinary FLC excretions.

Methods: Fresh paired samples of serum and 24h urine were analyzed in 270 patients exempt of monoclonal gammopathy. Patients with H (n=87) had sum of serum Ig G, A and M (Σ Ig) concentrations ≥ 20 g/L. All patients were classified in 6 groups according to their renal function and the presence, or not, of H; Control patients (C, HC) had physiologic proteinuria (<150 mg/24h) and serum creatinine concentration in reference ranges. Additionally, control patients C had serum K and L concentrations and rFLC values in the 100% reference interval. Patients with predominant tubular (T, HT) or glomerular (G, HG) proteinuria were determined by SDS-AGE profil (Hydragel Proteinuria®, Sebia) and by albuminuria ≤ or ≥ 50% of total proteinuria, respectively. FLC renal clearance (Clr) was calculated as the ratio of 24h urinary excretion of K or L FLC (mg/24h) to their respective serum concentration (mg/L).

Results: median (ranges); Mann-Withney test (significance: P<0.05), Spearman correlations (significance: P<0.05)

Results: Both in patients with H and in those without, K and L FLC urinary excretions were significantly greater in T and HT and in G and HG patients than in their respective control patients C and HC; these values were up to 151 (7-574) and 35 (2-261) mg/24h, respectively in G patients, 421 (93-8640) and 96 (34-3270) mg/24h, respectively in HG patients. Moreover, FLC excretions significantly correlated with both serum FLC and creatinine concentrations; in addition, in patients with H, they significantly correlated with Σ Ig concentrations. Ratios of K Clr to L Clr (K Clr /L Clr) decreased significantly through different states of renal function from C to T and to G patients and were negatively correlated with serum creatinine and Σ Ig concentrations. Patients with H, as compared to those without at the same stage of renal function, had significant and similar increases in K and L FLC excretions (3.5 and 4.0 fold for K and L FLC, respectively). While K Clr/L Clr values were similar in C and HC patients, they were significantly lower in HT than in T patients and in HG than in G patients.

Conclusion: This study determined the appropriate reference intervals for patients with H and/or RI. In all these patients, urinary polyclonal FLC excretions varied according to renal function. Besides, H was associated with an increase in FLC excretions values that was independent of renal function. RI progression was associated with a decrease in K Clr/L Clr values showing that the renal capacity to clear K faster than L is progressively lost. In addition, this effect worsened in patients with H suggesting a polyclonal FLC-mediated injury.

A-031

Serum Free Light Chains in Patients With Polyclonal Hypergammaglobulinemia And/Or Renal Impairment

J. Wils[1], B. Legallicier[2], V. Brunel[1], M. Quillard[1], M. Godin[2], S. Claeyssens[3]. *[1]Medical Biochemistry, University Hospital, Rouen, France, [2]Nephrology, University Hospital, Rouen, France, [3]Medical Biochemistry, University Hospital - Inserm UMR107 - IRIB, Rouen, France*

Background: Serum immunoglobulin-free light chain (FLC) assay is a major marker in the identification and management of patients with plasma cell dyscrasias. However, since these patients frequently present renal impairment (RI), it should be interpreted with caution owing to the dependence of polyclonal FLC values on renal function. Besides, recent data reported that serum FLC concentrations increased in patients with polyclonal hypergammaglobulinemia (H); however, data were rare and renal function was not always assessed. In this study, we assessed the effect of H and/or RI on serum FLC concentrations.

Methods: Fresh paired samples of serum and 24h urine were analyzed in 270 patients exempt of monoclonal gammopathy. Patients with H (n=87) had sum of serum Ig G, A and M (Σ Ig) concentrations ≥ 20 g/L. All patients were classified in 6 groups according to their renal function and the presence, or not, of H. Control patients (C, HC) had physiologic proteinuria (<150 mg/24h) and serum creatinine concentration in reference ranges. Additionally, control patients C had serum K and L concentrations and rFLC values in the 100% reference interval. Patients with predominant tubular (T, HT) or glomerular (G, HG) proteinuria were determined by SDS-AGE profil (Hydragel Proteinuria®, Sebia) and by albuminuria ≤ or ≥ 50% of total proteinuria, respectively. Serum FLC (Freelite®, The Binding Site) were analysed on a BNII nephelometer (Siemens Healthcare). Results: median (ranges); Mann-Withney test (significance: P<0.05), Spearman correlations (significance: P<0.05).

Results: Both in patients with H and in those without, serum K and L FLC concentrations rose significantly through different states of renal function from C to T and to G patients; in those latter, these values reached 36 (9-115) and 36 (9-106) mg/l, respectively in G patients, 139 (40-945) and 109 (30-691) mg/l, respectively in HG patients. Moreover, FLC concentrations and rFLC values were significantly correlated with creatinine concentrations; in addition, in patients with H, FLC concentrations significantly correlated with Σ Ig concentrations. Patients with H, as compared to those without at the same state of renal function, had significant and similar increases in FLC concentrations (3.5 and 3.0 fold for K and L FLC, respectively, for comparisons

of C vs HC patients, T vs HT and G vs HG patients). Alike, patients with H had a significantly and similar increase in rFLC values than those without (1.2 fold for comparisons of C vs HC patients, T vs HT and G vs HG patients).

Conclusion: This study determined the appropriate reference intervals for patients with H and/or RI. In all these patients, serum polyclonal FLC concentrations and rFLC values shifted to higher values with RI progression and varied according to renal function. Besides, H was associated with an increase in FLC concentrations and in rFLC values that were independent of renal function. Therefore, rFLC values should be interpreted with caution, not only in case of RI, but also in case of H: we showed that rFLC values between 0.24 to 0.74 should provoke a thorough search for plasma cell dyscrasias and lymphoproliferative disease.

A-034

Huaier suppresses proliferation and induces apoptosis in human lung adenocarcinoma cells via promotion of miR-26b-5p

Z. Lu, T. Wu, W. Chen, S. Liu, H. Lu, H. Wang, X. Huang, Q. Kong. *Central Hospital of Wuhan, Wuhan, China*

Background: Aqueous extract of Trametes robiniophila murr(Huaier)has been applied for cancer complementary therapy in recent decades. Varies studies have reported that Huaier possess the anti-tumor effects. However, the mechanisms are not completely elucidated. MicroRNAs (miRNAs) are small (18-25 nucleotides), noncoding RNAs whose dysregulation have been discovered to involve in tumorigenesis and development.

Methods and Results: In this study, we found miRNA expression profiles were altered in Huaier-treated human pulmonary adenocarcinoma A549 cells. MiR-26b-5p, which is upregulated in the expression profiles and simultaneously downregulated in both several lung cancer cell lines and patients, was selected for further study. We then used miRNA mimics or inhibitors to perform gain- and loss-of-function studies to demonstrate the roles of miR-26b-5p in pulmonary cancer. Moreover, EZH2 was identified as a target of miR-26b-5p by luciferase reporter assay and by EZH2 knock-down we observed a decrease in cell proliferation and an increase in apoptosis rates of A549 cells, which was corresponding to the effects of both Huaier treatment and the transfection of miR-26b-5p mimic. Additionally,β-catenin and bcl-2, as the indirect downstream effectors of EZH2, was found attenuated after Huaier treatment and miR-26b-5p overexpression.

Conclusion: Taken together,our findings shed light on the mechanisms that Huaier might suppress proliferation and induce apoptosis in lung cancer by miR-26b-5p-EZH2-mediated approach in lung cancer cells, which provides a new idea for understanding the anti-tumor effects of Huaier.

A-035

Blood Test for Early Detection of Lung Cancer

M. Stengelin[1], H. Pass[2], W. Rom[2], S. Kumar[1], S. Vaithlingam[1], A. Aghvanyan[1], D. Roy[1], G. Dobrescu[1], R. Sivakamasundari[1], E. N. Glezer[1], J. N. Wohlstadter[1]. *[1]Meso Scale Diagnostics, LLC, Rockville, MD, [2]New York University, New York, NY*

Background: Lung cancer is the largest single cause of death from cancer worldwide. Even though lung cancer often can be treated successfully when detected early, approximately 90% of patients diagnosed with lung cancer die of the disease. Screening with low dose CT can reduce mortality, but the positive predictive value of this test is low, leading to a large number of suspicious but ultimately non-malignant results that nevertheless require follow-up. Our objective was to develop a simple blood test to risk-stratify patients at high risk of lung cancer.**Methods:** We developed multiplexed, serum/plasma immunoassay panels to measure more than 40 lung cancer-related biomarkers using a 96-well, 7-spot format and electrochemiluminescence detection. Due to the high sensitivity of MSD's MULTI-ARRAY® technology, these panels were run with diluted serum or plasma, bringing the total sample volume required to run all 40 assays down to approximately 40 µL per replicate. This enabled us to measure all markers simultaneously in precious, high-quality serum and EDTA plasma samples. We used samples from early-stage lung cancer patients (drawn before lung cancer surgery) and from a lung-cancer screening cohort of age-matched heavy smokers who did not have lung cancer at the time of the blood draw.**Results:** In a training set of 300 samples, 12 serum and 6 plasma markers had areas under an ROC curve (ROC areas) of 0.7 or higher. We used a logistic regression model with 100x cross-validation to develop a multi-marker panel. One serum panel (Flt-3L, EGFR, MMP-3, and NME-2) and one plasma panel (Flt-3L, cytokeratin-19, MMP-3, Flt-1, KGF, and PlGF) were selected and tested using approximately 250 additional samples from the same cohort. For the serum panel, the ROC area dropped to 0.85 (vs. 0.95 for the training set); for the plasma panel, the ROC area dropped to 0.81 (vs. 0.93). Nevertheless, even the ROC area of 0.85 for the serum panel with clinical sensitivity and specificity of 81% and 84%, respectively, and the ROC area of 0.81 for the plasma panel with clinical sensitivity and specificity of 76% and 78%, respectively, should be clinically useful. Analysis of the combined training and test sets with 100x cross-validation resulted in a 4-marker serum panel (Flt-3L, EGFR, MMP-3, and NME-2) with an ROC area of 0.91 and clinical sensitivity and specificity of 88% and 82%, respectively, and a 5-marker plasma panel (Flt-3L, cytokeratin-19, Flt-1, KGF, and HGF) with an ROC area of 0.91 and clinical sensitivity and specificity of 84% and 83%, respectively. **Conclusion:**Using MULTI-ARRAY technology and high quality clinical samples, we were able to identify promising biomarker panels for early detection of lung cancer in high-risk individuals.

A-036

Serum MicroRNA Panel as Novel Non-Invasive Biomarker for Early Diagnosis of Cervical Cancer

Y. Zhang[1], N. Ning[1], Q. Li[2], W. Cui[1]. *[1]Peking Union Medical College Hospital Chinese Academy of Medical Sciences, Beijing, China, [2]Duke University Medical Center, Durham, NC*

Background: Currently, pathologic evidence of malignant cells, which typically requires an invasive strategy such as vaginoscopy and cervical biopsy, or loop electrosurgical excisional procedure, is referenced as the gold standard in diagnosing human cervical cancer. And serum tumor biomarkers, such as CEA, AFP, CA125, SCCAg, have provided some predictive information to tumor diagnosis, but poor sensitivity and specificity has also limited their clinical applications. We aimed to identify serum miRNAs for diagnosing cervical cancer.

Methods: Serum miRNA expression was investigated from 348 participants by using qRT-PCR, including 111 patients with cervical cancer, 115 cervical intraepithelial neoplasia (CIN) individuals and 122 healthy controls, recruited between July 2012 and November 2013 from Peking Union Medical College Hospital in China. First, we fully screened the differently expressed 425 miRNAs in 9 serum samples for diagnosing cervical cancer. A logistic regression model was constructed using a training cohort (n=72) and then validated using an independent cohort (n=269). Hela cells stably expressing miR-497 were established to analyze their roles in vivo and vitro.

Results: We identified a miRNAs panel (miR-124-3p, miR-195, miR-2861, miR-497) that provided high accuracy in discriminating cervical cancer from healthy controls (AUC=0.907 and 0.793 for training and validation groups, respectively), from CIN individuals (AUC=0.960 and 0.963 for training and validation groups, respectively). This 4 miRNAs can also differentiate CIN from healthy controls (AUC=0.903 and 0.87 for training and validation groups, respectively). Among the 4 up-regulated miRNAs, miR-497 levels in serum were the most specific one for cervical cancer that had no significance between ovarian or breast cancer patients and healthy controls. Forced expression of miR-497 suppressed proliferation and induced apoptosis of Hela cells ($p<0.05$) in vitro. Further investigation showed that Hela xenograft mouse treated miR-497 overexpression was significantly smaller in weight than control ($p<0.05$). MiR-497 could exert the effect of tumor growth inhibition in vivo.

Conclusion: Our results have identified a miRNAs panel (miR-124-3p, miR-195, miR-2861, miR-497) that has considerable clinical value in diagnosing cervical cancer as a novel noninvasive approach.

Keywords: miRNA panel; cervical cancer; serum

A-038

Can the incomplete serum separation on gel tube vacutainers lead to the diagnosis of Multiple Myeloma ?

S. Sen[1], S. Chakraborty[2]. *[1]Calcutta Medical Research Institute (CMRI), Kolkata, India, [2]Peerless Hospital & B K Roy Research Centre, Kolkata, India*

BACKGROUND: Gel tubes have become common in clinical labs and have made analysis and storage of samples easier; eliminating the need for transferring of serum into secondary tubes The basic principle is gradient density centrifugation using the thixotropic property of the gel, where it forms a barrier separating serum from the cells. Occasionally incomplete separation is seen in some samples where the gel packed cells fail to go below the gel. There are very few studies conducted on this phenomenon and some studies have suggested that incomplete separation of serum occur in patients with paraproteinemia particularly multiple myeloma (MM).

METHODS: Hence we conducted a prospective study on the relationship between incomplete serum separation on gel tubes and paraproteinemia. This was done to identify whether incomplete gel separation was associated with increased total protein. The gel tubes used in our study was BD- SST.

RESULTS: This study was done for a period of two years. Incomplete gel separation was seen in a total 14 gel tube samples out of a total of 99,850 patient samples (0.010 %).In 4 samples the incomplete separation was corrected on repeat sample collection. In 10 patient samples we observed incomplete separation even on repeat sampling. Raised total protein with altered albumin to globulin ratio was seen in those samples. Serum Protein Electrophoresis (SPEP) confirmed the presence of M bands in all the 10 cases and subsequently multiple myeloma was confirmed with bone marrow aspiration. The immunoglobulin subtypes with immunofixation were: Ig A (5/10), Ig M (4/10) and biclonal type with Ig M and Ig G (1/10).

CONCLUSIONS: Our study shows that incomplete separation on gel tubes is very commonly associated with paraproteinemia like multiple myeloma. The increase in paraprotein component possibly increases the viscosity of the sample leading to inhibition in separation. Clinical laboratorians need to be aware of this and should estimate the total protein and albumin reflectively in those patients showing incomplete separation. Patients having increased total protein and altered albumin globulin ratio should be followed up with SPEP. Clinical correlations and interaction with treating physicians might lead to early diagnosis in such patients

A-039

Clinical comparisons of two free light chain assays to immunofixation electrophoresis for detecting monoclonal gammopathy

M. Park, H. Kim, H. Kim, K. Shin, W. Song, H. Kim, H. Kim. *Hallym University College of Medicine, Seoul, Korea, Republic of*

Background: Free light chains (FLC) are useful biomarkers for the diagnosis and monitoring of various plasma cell dyscrasias. Recently, several monoclonal antibody-based assays for serum FLC have become available.

Methods: One hundred fifty seven samples from 120 patients for screening or monitoring of monoclonal gammopathy (MG) were included in this study. The new N Latex FLC assays (Siemens Healthcare Diagnostics GmbH, Germany) were compared with the Freelite FLC assays (The Binding Site Ltd, UK) and immunofixation electrophoresis (IFE).

Results: The Freelite FLC assay showed significantly wider assay ranges than the N Latex FLC assay. The correlation coefficients of the two FLC kappa (κ) assays, lambda (λ) assays, and the κ/λ ratio were 0.9792, 0.8264, and 0.9064, respectively. The concordance rate was 84.7% for the FLC κ assays, 79.6% for FLC λ, and 89.2% for the κ/λ ratio. Compared to the results for IFE, the clinical sensitivity, specificity, and percent agreement of the κ/λ ratios were as follows: 72.2%, 93.6%, and 82.8%, respectively, for the Freelite assay and 64.6%, 100%, and 82.2%, respectively, for the N Latex FLC assay. **Conclusion:**

Several differences in dynamic assay ranges were observed between the two FLC assays. The N Latex FLC assay showed good correlations and concordance with the Freelite FLC assay. The clinical sensitivity of the κ/λ ratio was higher in the Freelite FLC assays; however, clinical specificity was higher in the N Latex FLC assay.

A-040

Contribution of FokI polymorphism to disease development and risk prediction values in bladder cancer cases

O. Baykan[1], M. Akgul[1], N. Uren[2], F. Gerin[1], I. Tınay[1], E. Ergul[1], A. Sazci[2], L. Turkeri[1], G. Haklar[1]. *[1]Marmara University School of Medicine, Istanbul, Turkey, [2]University of Kocaeli, Kocaeli, Turkey*

Bladder cancer is the fourth most common cancer in men. Although smoking is known to be the most important etiological factor in bladder cancer, 40% of cases remained to be unknown. In our study we aimed to investigate the contribution of a common single nucleotide polymorphisms rs2228570 (FokI) in the vitamin D receptor gene to the formation of urothelial bladder cancer.

Age and gender matched 101 patients diagnosed as urothelial bladder cancer and 109 healthy individuals who has no history of cancer in their first degree relative were included in the study. Polymerase chain reaction, and restriction fragment lenght polymorphism techniques were used to determine the polymorphisms. The frequencies of FokI polymorphism FF, Ff and ff genotypes were 60.4%, 31.7%, 7.9% in bladder cancer and 44%, 47.7% and 8.3% in controls, respectively (p=0.048). FF genotype frequences were higher (p=0.018) in patients, while Ff frequences were lower (p=0.018) compared to controls (Table 1).

Table 1. Genotypic distribution of FokI polymorphism

VDR gene	Patient (n=101)	Control (n=109)	OR (%95 GA)	p	OR_{adj}* (%95 GA)	p
FF	61 (60.4%)	48 (44.0%)	1.94 (1.12–3.36)	0.018[a]	1.64 (0.89–3.02)	0.114[a]
Ff	32 (31.7%)	52 (47.7%)	0.51 (0.29–0.89)	0.018[b]	0.63 (0.34–1,19)	0.154[b]
ff	8 (7.9%)	9 (8.3%)	0.96 (0.35–2.58)	0.929[c]	0.82 (0.27–2.48)	0.727[c]

[a]FF vs.Ff+ff; [b]Ff vs. FF+ff; [c]ff vs. FF+Ff

*OR (95% CI) adjusted according to smoking history

Associations between risk factors and cancer were estimated by calculating OR_{adj} using logistic regression analyses. When smoking status and FokI polymorphism analysed together, the effect of genotype and allel frequencies on cancer risk prediction were not statistically significant, however smoking increased bladder cancer risk 7.27 times (OR_{adj}= 7.27; 95% CI= 3.8-13.9; p<0.001) The genotype distributions of the polymorphisms were in agreement with Hardy-Weinberg equilibrium among the cases and controls.

Studies investigating the contribution of VDR gene polymorphism and urothelial cancers were limited, although there were many publications for other cancer types. Our study is the first for inverstigating this relation in Turkish population. We demonstrated significant polymorphism in the patient group when compared to the control, however there was no effect of genotype on cancer occurence. Further studies which will be planned to reveal the effect of this difference may be beneficial in the etiopathogenesis of urothelial cancers.

A-041

Enrichment of heterogenous circulating tumor cells by multiplexed immunomagnetic micro particles

K. Bourcy[1], K. Goudy[1], L. M. Millner[2], M. W. Linder[1], R. Valdes[1]. *[1]PGXL Technologies, Louisville, KY, [2]University of Louisville, Louisville, KY*

Background: Circulating tumor cells (CTCs) are low abundance cells that have detached from the primary tumor and may produce metastatic lesions. They represent the greatest threat to a cancer patient. Paradoxically, they may also be an invaluable source of prognostic information by predicting metastases and chemotherapy resistance. Significant obstacles and limitations exist in current methods utilized for the detection and isolation of CTCs. Currently, the only FDA-cleared system for the detection of CTCs has a number of drawbacks including: i) the requirement that buffy coat be used, resulting in cell loss; ii) the cells must be fixed, which severely limits the ability to perform downstream analysis; and, iii) this system uses a single antigen for the recovery of CTCs, which can miss cells that do not express the target antigen. In this study we describe the application of a magnetic bead technology for enrichment of low abundance CTC where we evaluated this technology for enrichment of low abundance cells from whole blood, potential benefits in enrichment using a multi-antigen approach, and viability of CTCs following the enrichment procedure. *The long-term objective of this study is to develop a multi-plex immunomagnetic method for the isolation and recovery of circulating tumor cells from whole blood, with the ultimate goal of characterizing circulating tumor cells in breast cancer patients*.

Methods: We pre-labeled SKBR cells with carboxyfluorescein succinimidyl ester and spiked 10 or fewer cells into 1 mL of whole blood. The spiked blood was incubated with anti-EpCAM or a combination of anti-EpCAM and anti-HER2 conjugated magnetic microbeads. Blood was applied to a magnetized separation column and washed 4x with buffer. Magnetically labeled cells are retained in the column while unlabeled cells pass through. The column was removed from the magnet and labeled cells were eluted with buffer, sedimented by centrifugation and resuspended in 10 µL of elution buffer. We visualized and quantified cells using a fluorescent microscope and cell viability was determined based on a Trypan blue exclusion. All procedures were performed at room temperature.

Results: The average percent recovery from 1 mL of blood using anti-EpCAM-conjugated beads was 70.95% ± 5.27 (mean ± SEM), interassay CV of 40.68% (N=30). The average percent recovery from 1 mL of blood using a combination of anti-EpCAM and anti-HER2 beads improved to 81.32% ± 3.959 and interassay CV of 24.82% (N=26), p = 0.1308. The data was analyzed using an unpaired t-test. For each method of cell recovery, 100% of CTCs remained viable.

Conclusion: Based on these data, we concluded that this technology can be adapted for the purpose of enriching low abundance cells directly from whole blood. Use

of a multiplex strategy has the potential to achieve recovery efficiency consistent with the current state of the art, and improves % recovery over a single antigen approach. This method consistently yielded recovery of viable cells which will make this approach uniquely useful for single cell phenotyping studies such as for testing chemotherapeutic sensitivity.

A-042

Decoding miRNA Expression of Breast Carcinoma Behavior using Next Generation Sequencing of LCM Procured Cells

J. L. Wittliff[1], K. Bramlett[2], S. A. Andres[1], J. Schageman[2], C. Hinahon[2], J. Cienfuegos[2], R. Setterquist[2]. *[1]University of Louisville, Louisville, KY, [2]Thermo Fisher Scientific, Austin, TX*

Refinements in selecting biomarkers for breast carcinoma management require identification of clinically relevant parameters complementing patient endocrine status and tissue biopsy content of estrogen receptors (ER), progestin receptors (PR) and HER-2/neu oncoprotein, which correlate with prognosis and therapy response. Our objective is to compare miRNA expression profiles of intact tissue sections from breast cancers with those of laser capture microdissection (LCM)-procured carcinoma cells. The hypothesis is that miRNA signatures, discerned from LCM acquired populations of specific cell types, more accurately reflect the molecular basis of cancer clinical behavior than provided by protein biomarkers of intact tissue biopsies. miRNAs are 19-24 nucleotide non-coding RNA species regulating gene expression by inhibiting translation or by triggering degradation of specific mRNA targets. De-identified frozen tissue biopsies were selected from our IRB-approved Biorepository using criteria in the comprehensive de-identified Database to standardize the study population (e.g., invasive ductal carcinomas of known grade and biomarker status). Serial tissue sections containing 55 +/- 23% cancer were prepared and stained with H & E using established protocols, and carcinoma cells (~ 14000 LCM pulses) were procured non-destructively from an adjacent section. RNA was extracted from intact tissue sections and LCM-isolated cells using PureLink RNA Mini KitsTM (Invitrogen), evaluated for integrity (Agilent Bioanalyzer) and analyzed for miRNA expression using the Ion TorrentTM Next Generation Sequencing System (Thermo Fisher Scientific). Total RNAs were enriched for small RNA species using mirVana miRNA isolation kits (Thermo Fisher Scientific) and RNA libraries were constructed from 5 ng of enriched RNA using Ion Total RNA-Seq LibraryTM kits. Barcodes were utilized to multiplex libraries for template preparation and sequencing on two Proton PI chips as two twelve-plex library pools. Resulting sequences were aligned to mirBASE precursors, and expression levels were calculated by tallying the number of reads mapping to each individual miRNA precursor. Counts/1M reads values for each miRNA were normalized to a housekeeping gene to determine relative miRNA expression. Reads mapping to mirBase were assessed for each carcinoma preparation. Comparison of the top 20 expressed miRNAs in the intact tissue sections with those of cognate carcinoma cells procured by LCM, in general, revealed that smaller defined miRNA gene sets were expressed in isolated populations of carcinoma cells. Furthermore, miRNA expression patterns of experimental pairs (intact section vs LCM-procured cells) were highly variable in carcinomas with different grades, suggesting relationships to disease status. Strikingly, when miRNA gene frequency plots (transcript abundance vs fold-change) were developed, comparing expression from intact tissue sections to that of LCM-procured cell population, subsets of miRNA genes were revealed. Although the limited number of samples analyzed precluded identification of particular miRNA gene signatures associated with a specific breast pathology or biomarker status (e.g., ER, PR, HER-2/neu), application of Next Generation Sequencing of miRNAs using LCM-procured carcinoma cells provides an innovative approach for decoding miRNAs involved in breast cancer behavior. Supported in part by a grant from the Phi Beta Psi Charity Trust (JLW & SAA) and a CTSP Award from the Commonwealth of Kentucky (JLW).

A-044

Novel Approach to Diagnose High Grade of Cervical Lesion: Combination of HPV E6/E7 and hTERT mRNA Real-Time RT-PCR Assay

S. Park[1], H. Wang[2], S. Kim[3], G. Kim[1], D. Lee[4], Y. Kim[1], H. Kim[1], J. Kim[1], S. Ahn[1], H. Jin[5], K. Park[6], H. Lee[1]. *[1]Department of Biomedical Laboratory Science, College of Health Sciences, Yonsei University, Wonju, Korea, Republic of, [2]M&D, Inc., Wonju Eco Environmental Technology Center, Wonju, Korea, Republic of, [3]Institute for Life Science and Biotechnology, Yonsei University, Seoul, Korea, Republic of, [4]Department of Clinical Laboratory Science, Hyejeon College, Hongseoung, Korea, Republic of, [5]Department of Clinical Laboratory Science, College of Health Sciences, Catholic University of Pusan, Busan, Korea, Republic of, [6]Department of Pathology, Yonsei University Wonju College of Medicine, Wonju, Korea, Republic of*

Background: Human Papillomavirus (HPV) is a major causative factor of cervical cancer, which is the third of the most common cancer in women. The Real-HPV-E6/E7 mRNA® multiplex RT-qPCR assay (M&D, Wonju, Republic of Korea) has been developed and evaluated because E6 oncoprotein inhibits apoptosis by degradation of cellular tumor suppressor protein p53 and E7 oncoprotein prevents cell cycle arrest of damaged cells. HPV high-risk types (HPV genotype 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68 and 69) are regard to be detectable and significant marker in high grades of cervical lesion. Human telomerase reverse transcriptase (hTERT) is also considered as complementary marker that may provide a criteria in high grades of cervical lesions. Aberrant telomerase activity has been suggested to be critical for human tumor genesis and related to following mechanism of E6 oncoprotein.

Methods: In this study, HPV E6/E7 oncogene and hTERT are detected by Real-HPV-E6/E7 mRNA® multiplex RT-qPCR and Real-hTERT mRNA® RT-qPCR assay (M&D), respectively. A total of 545 patients including 18 squamous cell carcinoma (SCC), 21 high grade squamous intraepithelial lesion (HSIL), 17 atypical squamous cells-cannot exclude HSIL (ASC-H), 101 low grade squamous intraepithelial lesion (LSIL), 100 atypical squamous cells of undetermined significance (ASC-US), and 288 normal cytology samples, were enrolled and analyzed by cytological diagnostic grades, respectively. 39 samples in high grades of cytological diagnosis (≥ HSIL) were confirmed as high grades in histological diagnosis.

Results: The positive rates of HPV E6/E7 mRNA RT-qPCR assay were 94.4% (17/18), 95.2% (20/21), 82.4% (14/17), 46.5% (47/101), 25.0% (25/100), and 1.1% (3/286) in SCC, HSIL, ASC-H, ASC-US, LSIL, and normal samples, respectively. Relative hTERT mRNA expression levels were able to distinguish high grade and low grade of cervical lesion significantly (p <0.001). Relative hTERT mRNA expression levels in low grades of cervical lesion were dramatically lower than in high grade of cervical lesion. Notably, 5 high grades of cervical samples (≥ HSIL) were not detected by HPV E6/E7 mRNA real-time RT-PCR assay, but those samples were high relative expression levels with hTERT mRNA real-time RT-PCR assay.

Conclusion: For predicting the outcomes of cervical intraepithelial neoplasia (CIN) 1 or CIN 2 patients, the combined use of HPV E6/E7 and hTERT mRNA RT-qPCR assay could be a significantly complementary approach for diagnosing high grade cervical lesions because of the hTERT mRNA expression levels highly increased in cervical cancer and which was very low in low grade cervical lesions and normal tissues. Therefore the combined detection of HPV and human factors as a predictive marker might be a very useful for monitoring of patients who have low grade of cervical lesions.

A-045

SENTIFIT®-FOB Gold® latex Fecal Immunoassay Test (FIT) evaluation on SENTiFIT®270 analyzer in CoreLab at the AUSL Modena-Nuovo S.Agostino Estense hospital in Emilia Romagna Region.

M. C. Anelli[1], A. R. Soliera[2], N. Conti[1], A. Cugini[1], T. Trenti[2], F. Torricelli[2], R. Corradini[2], M. Gramegna[1]. *[1]Sentinel CH SpA, Milano, Italy, [2]CoreLab, Clinical Pathology Department, NOCSAE-AUSL Modena, Baggiovara, Italy*

Background: the fecal immunochemical test (FIT) for hemoglobin is considered to be superior guaiac fecal occult blood test for colorectal cancer (CRC) screening and is becoming central in international CRC screening programs development. Identify the most appropriate FIT is now a priority. The focus of this study is two-fold: to assess the FOBGold® Latex reagent FIT on a dedicated instrument with pierceable device; to compare two quantitative FITs.

Methodology: analytical evaluation: Limit of Blank (LoB) and of Quantitation (LoQ),

(CLSI EP17-A); total, between days and runs imprecision (EP5-A2) on 3 quality control levels (QC Low, 1, 2) and one hemoglobin spiked pool in buffer (target 50, 77, 309 and around 150 ng/mL respectively); linearity (EP6-A), prozone, on-board reagent and calibration stability (EP25-A).

Methods: 120 selected samples from frozen anonymous routine (not from CRC screening program population) residuals, with high positive prevalence, sampled with Eiken and Sentinel devices (OC-Auto Sampling Bottle3 and SENTiFIT®pierceTube) and run with OC-Sensor Diana and SENTiFIT®270 respectively; statistical analysis: concordance table. Analyzer evaluation: one week familiarization; piercing, sample barcode reading, timing evaluation. Training and rating questionnaire (from 1 to 5 where 1=very poor, 3=neither good nor poor, 5=very good) to 18 technicians/degrees.

Results: LoB 4.1 ng/mL; LoQ 15.4 ng/mL. QC Low total imprecision: CV=6.2%, SD=3.0; between days CV=3.4%, SD=1.7; between runs CV=0.8%, SD=0.4. QC 1 total imprecision: CV=4.0%, SD=3.4; between days CV=0.2%, SD=0.2; between runs CV=1.5%, SD=1.3. QC 2 total imprecision: CV=2.1%, SD=7.5; between days CV=0.9%, SD=3.1; between runs CV:0.8%, SD=2.8. Pool total imprecision: CV=2.7%, SD=4.2; between days CV=1.2%, SD=1.8; between runs CV=0.4%, SD=0.7. Accuracy: Recovery QC Low 98%, QC1 111.7%, QC2 113.7%, Pool 102.7%. Linearity up to 871 ng/mL; prozone checked-up to 50,000 ng/mL; on-board reagent and calibration stability 33 days. Method comparison: Hamza et al. published a cut-off value of 117 ng/mL for FOBGold corresponding to 100 ng/mL for OC-Sensor. On this basis we evaluate the concordance: 99 results were negative and 16 positive with both methods; 5 results were positive with FOBGold only. Positive rate in the evaluated samples: OC-Sensor=13.3% and FOB Gold=17.5%. FOB Gold had higher positive rate than OC-Sensor (24%). SENTiFIT270: Familiarization was done successfully and the instrument is user friendly and reliable. Piercing and barcode reading on 200 tubes were always correct; time to first result: 14 minutes. Questionnaire rating mean values for software, sample management, calibration, quality control and maintenance are 4.1/4.2/4.0/4.1/4.8 respectively.

Conclusion: FOBGold Latex reagent FIT on SENTiFIT270 analyzer shows some important features: very low LoB, a Low QC at 50 ng/mL, 1250 on-bard test autonomy and automated maintenance; on-board samples capability should be improved. A regression study is not appropriate, due to sampling bias (different devices, different buffers) and different signal origin (different calibration materials and wavelenghts between OC-Sensor and SENTiFIT270) therefore a concordance table is the only possible statistical analysis. The discrepant samples cannot be investigated in this study with anonymous samples selected from routine with high positive prevalence, as it is clear that a final clinical validation needs colonoscopy check.

A-046

Prostate Specific mRNAs as Potential Specific Markers of Circulating Tumor Cells and for Detection of Prostate Cancer

W. Zhang, Z. Wang, E. Klein, M. K. Gupta. *Cleveland Clinic Foundation, Cleveland, OH*

Background: Currently, prostate cancer (PCa) screening relies on prostate specific antigen for early-detection of PCa. However, this test suffers with poor specificity with high rate of negative biopsy stressing the necessity of more specific biomarkers. Prostate-specific RNA markers can be used to detect circulating cancer cells in peripheral blood to provide a sensitive and specific alternative to detect PCa. We performed a pilot study to explore the role of a combined RNA markers including prostate specific membrane antigen (PSMA), prostate cancer antigen 3 (PCA3) and TMPRSS2-ERG fusion gene (TM-ERG) in screening for PCa.

Methods: Total RNA was extracted from peripheral blood mononuclear cells (PBMC) and was analyzed for PSMA, PCA3 and TM-ERG transcripts using quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR). The assays were calibrated using either purified RNA from PCa cell line (VCap, CRL-2876) or synthesized complemented RNA. Analytic sensitivity was tested on RNA extracted from spiked female blood with VCap. Total of 33 patients with PCa before treatment, and 19 age-matched male patients with no PCa were analyzed for all three markers.

Results: Based on experiments using VCap spiked female blood PSMA and TM-ERG mRNA were reliably detected at 1-10 cell/ml respectively. The median (95percentile) mRNA levels in patients with PCa were significantly higher for all three markers than benign controls ($p \leq 0.001$). Furthermore, PCa patients with surgical margin(+) had significantly higher levels than margin(-) patients. Combination of all three markers significantly increased the clinical sensitivity and specificity for PCa as evidenced by logistic regression analysis. Results are summarized in Table 1.

Conclusion: Measurement of PSMA, PCA3 and TM-ERG transcripts extracted from PBMC increases the detection rate of PCa, especially invasive PCa with high specificity. Further studies with large cohort are needed for clinical validation and to assess pathologic stage prior to radical prostatectomy.

Table 1: Quantitation of mRNA for PSMA, PCA3,TM-ERG in Blood

	PSMA	PCA3[a]	TM-ERG	LR[c]
Benign				
Median (95% CI)	0.8 (0.2-1.5)	256 (128-1020)	9.7 (7.0-12)	NA
PCa				
Median (95% CI)	2.8 (1.6-9.2)	3049 (1947-4988)	51 (15-81)	NA
p-value[b]	0.0001	<0.0001	0.0001	NA
Margin(+)(N=13)	17 (1.3-24)	4355 (2839-14631)	81 (61-111)	NA
Margin(-)(N=20)	2.2 (1.5-4.0)	2016 (832-5003)	17 (8.0-52)	NA
p-value	0.021	0.019	0.003	NA
ROC Analysis				
AUC (%)	83	88	78	96
p-value[d]	*0.004*	*0.090*	*0.001*	NA
Sensitivity (%)	76	76	68	85
Specificity (%)	79	90	100	95
[a]PSMA, TM-ERG: ng/PCR, PCA3: copies/PCR	[b]Mann-Whitney test	[c]Logistic regression analysis	[d]LR compare to individual marker (McNemar)	

A-047

Utility of Stringent Complete Response in routine treatment of Multiple Myeloma patients with novel agents

J. L. García de Veas Silva, C. Bermudo Guitarte, R. Duro Millán, V. Sánchez Margalet, C. González Rodriguez. *Hospital Universitario Virgen Macarena, Sevilla, Spain*

Background: Normalization of serum free light chains (sFLC) ratio in patients with Multiple Myeloma (MM) achieving complete response (CR) may define a deeper degree of response after therapy than that defined by the CR criteria. The stringent CR (sCR) requires normalization of sFLC ratio and absence of clonal plasma cells in bone marrow in addition to the criteria for CR (Negative immunofixation of serum and urine, disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow). The aim of this study is to evaluate the prognostic utility of sCR in patients newly diagnosed with MM treated with novel agents in the routine practice.

Methods: Twenty three patients with MM (10 IgG MM, 5 IgA MM, 2 IgD MM and 6 Bence Jones MM) achieving CR after therapy with Bortezomib/Dexametasone were included in this study. Disease Free Survival (DFS or time after treatment where disease remains stable) was estimated by Kaplan-Meier method and compared by log-rank tests. Cox proportional hazard analysis was performed for multivariate analysis. Serum free light chains were measured by turbidimetry (Freelite) in a SPA PLUS analyzer (The Binding Site Group Ltd, Birmingham, UK) and immunofixation was performed in a HYDRASYS (Sebia, FR) analyzer.

Results: The median follow-up of the patients was 18 months (range 14-31 months). Eleven patients achieved CR and 12 patients achieved sCR. During the period of study there were 8 relapses, six in patients achieving CR and two in patients achieving sCR. The median DFS for patients achieving CR was 18 months and not reached for those achieving sCR. Patients achieving CR had a DFS rate of 24% compared with 75% for sCR (p=0.022). Results showed that achieving a sCR was an independent prognostic factor for survival (HR = 6.57; 95% CI, 1.09-39.80; vs CR; p = 0.039).

Conclusion: The presence of an altered sFLC ratio suggests the existence of a persistent clonal population that is secreting small amounts of monoclonal protein. Our results indicate that sCR represents a deeper response state compared with conventional CR which translates into a longer DFS. Despite the small cohort, analysis of sFLC ratio was able to identify a group of patients with more favorable prognosis and support its inclusion in the response criteria for MM patients treated with novel agents.

A-048

Determination of ROMA Score Performance Using the Roche Elecsys HE4 and CA 125 Immunoassays

M. A. Lasho, A. Algeciras-Schimnich. *Mayo Clinic, Rochester, MN*

Background: Ovarian cancer is the fifth-leading cause of death in women. Ovarian cancer symptoms are related to the presence of an adnexal mass and are often vague and unspecific. Treatment of women presenting with an adnexal mass and at high risk for ovarian cancer by specialized gynecologic oncologists has been shown to improve patient outcomes. The risk of ovarian malignancy algorithm (ROMA) is a calculation that incorporates the patient's serum concentrations of cancer antigen 125 (CA125) and human epididymis protein 4 (HE4) in conjunction with the menopausal status to calculate a predictive probability of finding epithelial ovarian cancer on surgery in women presenting with an adnexal mass. Women are classified as high-risk or low-risk for ovarian cancer. ROMA has only been validated using HE4 by enzyme immunoassay (EIA) in conjunction with Abbott ARCHITECT CA125 assay

or CA125 by EIA. This study evaluates the use of the Roche Elecsys HE4 and CA125 electrochemiluminescence immunoassay (ECLIA) for the calculation of the ROMA score.

Methods: Serum samples from 114 premenopausal females (75 benign gynecological conditions, 39 epithelial ovarian cancer [EOC]) and 93 postmenopausal females (56 benign gynecological conditions, 37 EOC) were included in the study. Benign gynecological conditions included cysts, cystadenomas, leiomyomas, myomas, or fibromas. Epithelial ovarian cancers included stages I through IV (stage I N=19, II N= 3, III N=45, IV N= 9). HE4 and CA125 were measured using the Roche Elecsys ECLIA on a Roche Cobas e601 instrument. Serum HE4 and CA125 concentrations and menopausal status were used to calculate the ROMA score. Equations used to calculate the ROMA score were as follow: Premenopausal Predictive Index (PI) = -12.0 + 2.38*LN[HE4] + 0.0626*LN[CA125]; Postmenopausal PI = -8.09 + 1.04*LN[HE4] + 0.732*LN[CA125]; and ROMA score = exp(PI) / [1 + exp(PI)] * 10. Receiver Operating Characteristic (ROC) curve analysis was used to determine optimal clinical cut-points and clinical specificity and sensitivity.

Results: In premenopausal women, the ROMA ROC curve area under the curve (AUC) was 0.95. A ROMA score equal or greater than 1.00 yielded 75% specificity and 95% sensitivity. Using the manufacturer suggested cut-point of equal or greater than 1.14 yielded 84% specificity and 95% sensitivity. In postmenopausal women, the ROMA ROC AUC was 0.94. A ROMA score equal or greater than 2.44 yielded 75% specificity and 95% sensitivity. Using the manufacturer suggested cut-point of equal or greater than 2.99 yielded 86% specificity and 92% sensitivity.

Conclusion: This study established the performance of ROMA score cut-points using the Roche Elecsys HE4 and CA125 immunoassays. This information could serve as guidance for laboratories implementing the ROMA score in clinical practice.

A-049

Comparing the Performance of Newly Developed Heavy Chain/ Light Chain Immunoassays with Serum Protein Electrophoresis and Nephelometric Measurements of Total Immunoglobulin for Monitoring Multiple Myeloma Patients

L. Adie, O. Berlanga, H. Carr-Smith, S. Harding. *The Binding Site Group Ltd, Birmingham, United Kingdom*

Background: Both serum protein electrophoresis (SPEP) and total immunoglobulin (tIg) measurements have been recommended for quantification of monoclonal Ig (M-Ig). However, SPEP is inaccurate at low (<10 g/L) and due to dye saturations at high (>20-30 g/L) concentrations of M-Ig. By contrast tIg measurement is an accurate method but is unable to distinguish between monoclonal and polyclonal Ig. Newly developed Heavy chain/ light chain immunoassays may provide an alternative method of quantifying M-Ig concentrations. Here, we compare the performance of these assays with traditional methods for monitoring MM patients. **Methods:** HLC Ig'κ and Ig'λ were quantified in 127 IgG (87 IgGκ, 40 IgGλ) and 61 IgA (37 IgAκ, 24 IgAλ) MM patient sera. The results were compared to published normal ranges (IgGκ: 4.03-9.78 g/L, IgGλ: 1.97-5.71 g/L, IgGκ/ IgGλ: 0.98-2.75; IgAκ: 0.48-2.82 g/L, IgAλ: 0.36-1.98 g/L, IgAκ/ IgAλ: 0.80-2.04), historic SPEP, immunofixation and tIg concentrations. Weighted Kappa and Pearson correlation were used to analyse results. **Results:** At presentation all 127 IgG and 61 IgA patients had an abnormal HLC ratio and involved HLC (iHLC) concentrations (median (range) IgGκ: ratio 56 (6- 1275), iHLC 32 g/L (14-102); IgGλ: ratio 0.024 (0.001- 0.329), iHLC 34 g/L (9- 90); IgAκ: ratio 233 (10- 6226), iHLC 34 g/L (6- 79); IgAλ: ratio 0.0119 (0.0003- 0.1181), iHLC 29 g/L (6- 72)). Whilst M-Ig concentrations were measurable by SPEP in all IgG patients, only 66% (40/61) IgA patients were quantifiable. In all samples, iHLC and dHLC (involved HLC-uninvolved HLC) concentrations showed a good correlation with SPEP for IgG (iHLC y=0.83x+1.8, R^2=0.87; dHLC y=0.84x+0.48, R^2=0.88), IgA (iHLC y=0.88x+0.88, R^2=0.87; dHLC y=0.89x+0.44, R^2=0.88) and with tIgA measurement in IgA patients (iHLC y=0.87x-0.68, R^2=0.90; dHLC y=0.88x-1.33; R^2=0.90). During the course of the patients disease, changes in iHLC and dHLC concentrations reflected the changes in M-Ig measured by SPEP (IgG: iHLC y=0.87x-0.05, R^2=0.83; dHLC y=0.91x-0.06, R^2=0.86; IgA: iHLC y=1.31x+0.25, R^2=0.87; dHLC y=1.36x+0.28, R^2=0.88) and with tIgA changes in IgA patients (iHLC y=0.94x-0.03, R^2=0.90; dHLC y=0.96x-0.03, R^2=0.90). Responses assigned based on reductions in M-Ig measured by either iHLC, dHLC or traditional methods showed substantial agreement for IgG and near perfect agreement for IgA patients using Weighted Kappa analysis (IgG: iHLC vs. SPEP 81% agreement, Weighted Kappa (95% CI): 0.78 (0.56-1.00); dHLC vs. SPEP 80% agreement, Weighted Kappa (95%CI): 0.77 (0.55-1.00); IgA: iHLC vs. SPEP/tIgA 89% agreement, Weighted Kappa (95% CI): 0.92 (0.84-1.00); dHLC vs. SPEP/tIgA 89% agreement, Weighted Kappa 0.92 (0.84-1.00)). Changes in HLC ratio similarly showed a good comparison to the assigned responses (IgG: 71% agreement, Weighted Kappa (95% CI): 0.74 (0.56-0.92); IgA: 73% agreement, Weighted Kappa (95% CI): 0.86 (0.81-0.91)). **Conclusion:** Responses assigned using reductions in iHLC, dHLC, HLCr or SPEP showed a good agreement. Furthermore, iHLC, dHLC and HLC ratio were able to assign responses in 34% of IgA patients that were not quantifiable by SPEP. The HLC immunoassays provide an alternative method of quantifying M-Ig in patients with MM.

A-050

Serum MicroRNA Panel as Biomarkers for Early Diagnosis of Colorectal Adenocarcinoma

C. Wang, G. Zheng, L. Du, L. Wang, X. Zhang, Y. Yang, J. Li, Y. Wang, Y. Liang. *Qilu Hospital, Shandong University, Jinan, China*

Background: Due to the high mortality of colorectal adenocarcinoma (CAC), there is an urgent need to identify new biomarkers with high sensitivity and specificity. The recent discovery of serum microRNA (miRNA) profile in human cancer has provided a new auxiliary approach for tumor diagnosis. Our study is the first global analysis of serum miRNAs based on the normal-colorectal adenoma (CA)-CAC sequence.

Methods: Serum samples were collected from 307 CAC patients, 164 CA patients and 226 healthy controls. We firstly profiled pooled serum of CAC, CA and healthy controls by Miseq sequencing. The differentially expressed serum miRNAs were chosen as candidate biomarkers for CAC. Both the candidate reference genes and candidate biomarkers were validated by the reverse-transcription polymerase chain reaction (RT-qPCR). The miRNA panel was developed with a logistic regression model and then validated using an independent cohort. Receiver operating characteristic (ROC) curves were constructed, and area under the ROC curve (AUC) was used to evaluate the diagnostic accuracy of the panel.

Results: The Miseq sequencing results revealed 15 differentially expressed miRNAs in CAC patients compared with controls. Using the selected reference gene of miR-191-5p and U6, we identified a 4-miRNA panel (miR-19a-3p, miR-223-3p, miR-92a-3p and miR-422a) with a high diagnostic accuracy of CAC. Even in the low carcinoembryonic antigen (CEA) level group, the diagnostic accuracy of this miRNA panel was still acceptable (AUC = 0.810). Surprisingly, our results indicated that the miRNA panel could differentiate stage I/II CAC patients from controls. In addition, this panel could also differentiate CAC from CA (AUC=0.886).

Conclusions: In the present study, we established a serum miRNA panel with considerable clinical value in the early-stage diagnosis of CAC.

A-051

Total, free, and complexed prostate-specific antigen concentrations among U.S. men, 2007-2010

D. A. Lacher, J. P. Hughes. *National Center for Health Statistics, Hyattsville, MD*

Background: Screening for prostate cancer using prostate-specific antigen (PSA) is common but remains controversial. Prostate cancer has been associated with higher total PSA (tPSA), lower free PSA (fPSA), and lower percent free PSA (fPSA/tPSA x 100%). More recently, higher complexed PSA (cPSA), bound primarilywith α-1-antichymotrpsin, has been associated with prostate cancer. The distributions of total, free and complexed PSA concentrations, percent free PSA and percent complexed PSA (cPSA/tPSA x 100%), and the free/complexed PSA ratio in men were examined in the 2007-2010 National Health and Nutrition Examination

Survey (NHANES).

Methods: Total, free and complexed PSA were performed on 3251 men aged 40 years and older who were examined in the 2007-2010 National Health and Nutrition Examination Survey. NHANES is a cross-sectional, nationally representative, area probability survey of U.S. non-institutionalized participants. Distributions of the PSA tests were examined by age, race and ethnicity, and body mass index (BMI) groups. In addition, percentages of men at total and percent free PSA cut-points were examined. All PSA tests were log-normal in distribution except percent complexed PSA, which was normally distributed. The geometric mean (GM) or arithmetic mean (for percent complexed PSA), standard error (SE) of the mean, and selected percentiles were determined. Age-adjusted means were used for analysis of PSA tests for race and ethnicity and BMI groups.

Results: The geometric mean (SE) for tPSA was 0.96 (0.02) µg/L with 5.2% of men ≥ 4.0 µg/L and 1.1% ≥ 10.0 µg/L. Free PSA had a GM of 0.27 (0.01) µg/L. The GM of percent fPSA was 28.1 (0.3) % and 8.6% of men had ≤ 15% percent fPSA.

Complexed PSA had a GM of 0.53 (0.01) µg/L. The arithmetic mean of percent cPSA was 56.0 (0.5) % and the free/complexed PSA ratio GM was 0.52 (0.01). Total, free, and complexed PSA increased with age. Total PSA GM increased from 0.74 µg/L for men 40-49 years to 1.82 µg/L for men 80 years and older. Free PSA GM increased from 0.22 µg/L for men 40-49 years to 0.51 µg/L for men 80 years and older, while complexed PSA increased from 0.40 µg/L for men 40-49 years to 0.99 µg/L for men 80 years and older. The adjusted mean for non-Hispanic white men had lower tPSA (1.03 µg/L) and cPSA (0.56 µg/L) than non-Hispanic black men (tPSA 1.25 µg/L and cPSA 0.72 µg/L). Hispanic men had higher cPSA (0.64 µg/L) than non-Hispanic white men. Obese men had lower age-adjusted mean total, free and complexed PSA (0.94, 0.27, and 0.51 µg/L, respectively) than men with normal BMI (tPSA 1.21, fPSA 0.32, and cPSA 0.68 µg/L).

Conclusion: The free and complexed PSA may provide additional information in conjunction with total PSA in screening for prostate cancer. Total, free and complexed PSA increased with age; total and complexed PSA were highest in non-Hispanic black men; and obese men had the lowest total, free, and complexed PSA.

A-052

Leptin and insulin hormones increase Sam68 expression and phosphorylation in human breast adenocarcinoma cells

F. Sánchez-Jiménez, A. Pérez-Pérez, C. González-Rodríguez, J. A. Virizuela, V. SANCHEZ-MARGALET. *VIRGEN MACARENA UNIVERSITY HOSPITAL, SEVILLE, Spain*

Background: Obesity and insulin resistance are well known risk factors for breast cancer development in postmenopausal women. High insulin levels, together with other hormones, such as leptin and cytokines, IGFs, estrogen and EGF, positively modulate the growth of these tumor cells. All these factors may act through signaling cascades that lead to the final effect of increasing growth and cell proliferation. Sam68 protein is a member of the signal transduction activator of RNA (STAR) family of RNA-binding proteins that can interact both with RNA and signaling proteins. According to this dual role, Sam68 has been involved in different carcinogenic mechanisms including alternative splicing or cell cycle regulation. Moreover, our group has previously described the role of Sam68 in the insulin and leptin signaling pathways as a receptor substrate, and it has been shown to participate in proliferation, cellular growth and antiapoptotic effects mediated by these hormones in different cellular types.

Objective: We aim to study the expression of Sam68 and its phosphorylation level upon insulin and leptin stimulation, seeking for a possible role of Sam68 in leptin and insulin receptor signaling in human breast adenocarcinoma cells.

Methods: We used the human breast adenocarcinoma cell line MCF7. We studied leptin-mediated and also insulin-mediated Sam68 phosphorylation by immunoprecipitation and immunoblot with anti-phosphotyrosine antibodies as well as polyU affinity precipitation. Quantitative RT-PCR and immunoblot were used to study the effect of leptin and insulin on Sam68 expression. siRNA was used to downregulate Sam68 expression and its effects on leptin and insulin activation of MAPK and PI3K pathways. Phosphorylation of some of the main proteins of these pathways (ERK1/2 and MEK as well as PKB and P70s6K respectively) was tested by using immunoblot with antibodies against phosphorylated proteins and anti-tubulin as loading control.

Results: Sam68 protein quantity and gene expression were found to be increased under leptin as well as insulin stimulation, by using 1 nM dose after a 24 hours stimulus. Moreover, both insulin and leptin stimulation promoted an increase in Sam68 tyrosine phosphorylation in MCF7 cells and negatively regulated RNA binding of Sam68, as previously observed in other systems. Sam68 downregulation resulted in lower activation of MAPK and PI3K pathways under both hormones stimulation. Sam68 was necessary for the complete leptin and insulin phosphorylation of the main proteins of these pathways.

Conclusion: These results suggest the participation of Sam68 in both leptin and insulin receptor signaling in human breast cancer cells, where Sam68 could mediate the trophic effects of these hormones in proliferation and cellular growth. Thus, Sam68 could be also considered as a future prognostic marker or therapeutic target in this kind of non genetic breast cancer.

A-053

MiR-28-5p, a potential biomarker for renal cell carcinoma, acts as a tumor suppressor in renal cell carcinoma for multiple antitumor effects by targeting RAP1B

C. Zhang[1], C. Wang[1], C. Wang[1], C. Wu[1], Q. Yang[1], C. Zhang[2]. *[1]Jinling Hispital, Nanjing, China, [2]Nanjing Univertisy, Nanjing, China*

Background: The mechanisms involved in renal cell carcinoma (RCC) development and progression remain unclear, and new biomarkers are needed in routine practice to improve the diagnostic and/or prognostic accuracy. However, there is no standard serum biomarker to facilitate diagnosis or prognostic stratification in patients with RCC. There is increasing evidence that microRNAs (miRNAs) are involved in cancer development and progression and circulating miRNAs have great potential as biomarkers for diagnosis and prognosis in patients with several types of cancers. Our purpose was to investigate whether serum miR-28-5p could be a useful biomarker for the diagnosis of RCC and evaluate the functional significance of the miR-28-5p in RCC. **Methods:** This study included 33 RCC patients and 33 healthy controls. First, we analyzed tissue miR-28-5p levels in tumor tissues and matched normal tissues from the 33 RCC patients. Second, we investigated the serum miR-28-5p levels in the 33 RCC patients and the 33 normal controls. TaqMan probe based-RT-qPCR was used to measure serum miRNA levels. A combination of let-7d, let-7g and let-7i (let-7d/g/i) was used as endogenous control for normalizing the data of RT-qPCR. We also examined the expression level of miR-28-5p in some human RCC cell lines. The CCK8 proliferation, transwell and wound healing assays were used to explore the potential functions of miR-28-5p in RCC cells. Luciferase reporter assays were employed to validate regulation of a putative target of miR-28-5p. The effect of modulating miR-28-5p on endogenous levels of this target were subsequently confirmed via Western blotting. **Results:** MiR-28-5p expression was relatively decreased in RCC specimens compared with adjacent normal tissues ($P<0.01$). Consistent with the results from tissues, serum miR-28-5p levels were decreased in RCC patients compared to controls ($P<0.001$). ROC curve analysis showed an AUC of 0.90 (95% confidence interval, 0.85-0.95) and a sensitivity and specificity of 95 and 86%, respectively. MiR-28-5p was found to be also downregulated in human RCC cell lines A498 and Caki-2 as compared with normal cell line HK-2. Luciferase reporter assays showed that miR-28-5p directly regulated RAP1B. In RCC clinical specimens, the expression of RAP1B protein was significantly higher in cancer tissues than in non-cancerous tissues. Statistical analysis results indicated that the RAP1B protein level was negatively correlated to the miR-28-5p expression in RCC tissues ($P<0.05$). RAP1B protein was found to be upregulated in A498 and Caki-2 cells, and knockdown of RAP1B inhibited cell proliferation and migration, suggesting that RAP1B has oncogenic functions in RCC. Ectopic expression of miR-28-5p could result in increased RAP1B protein expressions, and inhibited proliferation and invasion of A498 and Caki-2 cells, while the downregulation of miR-28-5p with the inhibitor had the opposite effect. The miR-28-5p induced cell proliferation and migration could be rescued by RAP1B. **Conclusion:** MiR-28-5p may potentially serve as a novel biomarker for RCC and may act as a tumor suppressor in RCC progression by inhibiting the RCC cell proliferation and migration through targeting oncogeneRAP1B. Our findings indicate that targeting miR-28-5p by a genetic approach may provide a novel strategy for the treatment of RCC.

A-054

Serum thyroglobulin measurement in autoantibody positive samples by LC-MS/MS and immunoassay: is positivity rate different between the methods?

M. M. Kushnir[1], A. L. Rockwood[2], A. W. Meikle[3]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]Department of Pathology, University of Utah, Salt Lake City, UT, [3]Departments of Pathology and Medicine, University of Utah, Salt Lake City, UT*

Background: Measurement of thyroglobulin (Tg) in serum and plasma is used to monitor patients after treatment for differentiated thyroid carcinoma (DTC). A complicating factor in using Tg as biomarker of the recurrence of DTC is related to the presence of endogenous anti-Tg autoantibodies (Tg-AAb) in blood of many patients. Tg-AAb can interfere with immunoassay (IA) measurements and cause false-negative results. LC-MS/MS methods for Tg are expected to overcome Tg-AAb interference with measurement of Tg, but there were no studies supporting better utility of the LC-MS/MS methods for Tg in Tg-AAb positive samples.

Methods: Recently we developed LC-MS/MS method for measurement of Tg in serum samples (Clin Chem 2013;59:982-90). The lower limit of quantification is 0.5ng/mL; total imprecision is below 10%. We performed comparison of LC-MS/MS and Beckman IA using Tg-AAb negative and positive samples; and reviewed

historical data on analysis of Tg in Tg-AAb positive samples using Beckman IA (n=1367) and LC-MS/MS (n=6180) methods, by comparing Tg positivity rates for the assays. Tg calibrators were standardized between the methods. Measurement of Tg-AAb was performed by Beckman Antibody-II assay.

Results: In a set of Tg-AAb negative samples Tg concentrations determined with Beckman IA agreed well with LC-MS/MS (IA=1.00*LC-MS/MS-2.35, r=0.982, $S_{y.x}$=9.52); IA underestimated Tg concentrations in Tg-AAb positive samples. In a set of Tg-AAb positive samples tested negative for Tg using IA, concentrations determined by LC-MS/MS method were at or above 0.5ng/mL in 23% of samples. Positivity rate for different Tg cutoff concentrations and differences in the positivity rate between the methods are shown in Table.

Conclusions: Higher Tg positivity rate in Tg-AAb positive samples was observed by LC-MS/MS method as compared to the IA; the difference is likely caused by underestimation of Tg concentrations caused by interference of Tg-AAb with the IA.

Table. Tg positivity rate in Tg-AAb positive samples analyzed by LC-MS/MS and Beckman Coulter immunoassay.

	Percent of samples with concentration above the cutoff		
Tg cutoff concentration, ng/mL	LC-MS/MS, %	Beckman Access, %	Difference, %
>0.5	37.5	37.0	0.5
>0.6	37.1	34.8	2.3
>1	33.4	30.0	3.4
>2	26.4	23.9	2.5
>5	18.3	16.6	1.7
>7	15.1	13.8	1.3
>10	12.5	11.4	1.1
>15	10.1	9.2	0.9
>20	8.1	7.6	0.5

A-055

Value of a new biochemical parameter (serum Heavy chain/Light Chain pairs) in the follow-up of Multiple Myeloma after treatment

R. P. Garay, I. J. Ventura, E. A. Diez, E. Landeta, I. Amariko, A. G. Vicuña, A. S. Izarra. *Hospital Universitario Cruces, Bilbao, Spain*

Background:Multiple Myeloma (MM) has an incidence of 1-10% of all cancers. The laboratorial assays usually used for its diagnosis and follow-up are: detection of the monoclonal protein by Serum Protein Electrophoresis (SPE) and Immunofixation (IF) in serum and 24h urine, total immunoglobulins levels by nephelometry and serum Free Light Chains (sFLC). Determination of the bone marrow plasma cells, lytic lesions by MRI, complete blood count, creatinine and calcium levels are also used. Recently, a new technique that allows the analysis of immunoglobulin heavy chain/light chain pairs (Hevylite®) has been developed.Objectives:The sFLC determination has been introduced in 2006 in the Stringent Complete Response subcategory by the International Myeloma Working Group (IMWG), corresponding to a Complete Response plus normal FLC ratio and absence of clonal cells in bone marrow. The reasons for its inclusion are that it is a highly sensitive marker for sFLC and an excellent indicator of clonality (Durie et. al., Leukemia 2009). A recent study has further corroborated its correlation with a more stringent level of response with clinical prognostic impact (Kapoor et. al., JCO 2013). The aim of this study is to evaluate the utility of the Hevylite ratio (HLCr) together with sFLC in the follow-up of 3 MM IgG patients under treatment and follow-up.

Methods:sFLC and HLC were measured by turbidimetry (FreeliteTM and Hevylite®, on a SPAPLUS, Binding Site). The monoclonal protein was identified and quantified by electrophoresis (Capillarys Hydrasys Focusing, Sebia). Samples from 3 MM patients were analyzed:

-Patient 1: 58 years old woman with an IgG-L type MM stage II-A. 13 samples collected from June 2011 to July 2013. 1st line treatment (Velcade-Dexamethasone-Adriamycin) and TASPE (July 2012).

-Patient 2: 61 years old man with IgG-K MM stage II-A, ISS-2. 14 samples from May 2011 to November 2013. 1st line treatment (Velcade-Dexamethasone-Adriamycin) and TASPE (May 2012).

-Patient 3: 76 years old woman with IgG-K MM stage II, ISS-2. 14 samples from March 2013 until January 2014. 1st line treatment (Bortezomib-Melphalan-Prednisone) and 2nd line treatment (Cyclosphophamide-prednisone).

Results:-Patient 1: in sCR since December 2012. sFLC and HLC ratios normalized with absence of monoclonal protein by SPE and IF.

-Patient 2: achieved CR after TASPE, at this time in biochemical relapse. After treatment, the HLCr normalized in May 2012, the SPE became negative in August 2012 and the IF in November 2012, achieving a CR. The sFLC ratio is abnormal throughout follow-up, and currently there is a monoclonal protein detected by IF and SPE.

-Patient 3: Refractory MM with stable disease: abnormal sFLC and HLC ratios with 4.23 g/dL of monoclonal protein.

Conclusion:The inclusion of the Hevylite assay allows the quantitative follow-up of monoclonal immunoglobulins, particularly interesting when patients achieve a deepness of response where the standard SPE and IF become negative. Results obtained with Hevylite assay are in agreement with the other results. This preliminary data suggests that the new HLC assay may add specificity to the Stringent Complete Response. More studies are required to establish its prognostic value in the response evaluation.

A-056

Performance of the Roche Elecsys Thyroglobulin (Tg) II immunoassay

B. C. Netzel, M. A. Lasho, A. Algeciras-Schimnich. *Mayo Clinic, Rochester, MN*

Background: Serum thyroglobulin (Tg) measurement is considered the gold standard in the follow-up of patients with differentiated thyroid cancer following total thyroidectomy and radioactive iodine ablation. In athyrotic patients, Tg is an excellent tumor marker because it is produced exclusively by the follicular cells of the thyroid. Performance of currently available Tg immunoassays varies due to different assay sensitivities, standardization against the certified reference material (CRM-457), and Tg autoantibody (TgAb) interference, among others. In this study we evaluated the analytical performance of the Roche Elecsys Tg II immunoassay.

Methods: The Roche Elecsys Tg II immunoassay (Roche Diagnostics, Indianapolis, IN) is a quantitative, two step, double antigen sandwich assay standardized against CRM-457 for the measurement of thyroglobulin in serum and plasma using the electrochemiluminescence immunoassay "ECLIA" technology. The assay uses 35 µl of sample and has a total assay time of 18 minutes. Testing was performed on the Roche Diagnostics cobas e411 analyzer. Accuracy was investigated by recovery studies using CRM-457. Imprecision studies were conducted using Liquichek™ Tumor Marker Control (Bio-Rad, Hercules, CA) and Roche PreciControl quality control (QC) materials. Analyte measurement range (AMR) studies were conducted by diluting a high concentration Tg serum sample with a negative Tg serum sample. LOQ studies were conducted using a low concentration Tg serum sample. Method comparison studies with the Beckman Access Tg assay (Beckman Coulter, Brea, CA) and an in-house developed LC-MS/MS Tg assay was performed using de-identified serum samples collected for routine Tg determination.

Results: Average recovery of CRM-457 was 106% (range 101-111%). Inter-assay imprecision studies produced coefficient of variation (CV) of <6% (range 1.4-5.5%) at concentrations of 6.4, 21.8, 85 and 188 ng/mL. The AMR of the assay was 0.1 to 500 ng/mL with Passing-Bablock regression fit of y = 0.96x -0.32 (r^2=0.999). Serial dilutions (x2 to x64) to expand the AMR produced an average recovery of 97% (range 91-104%). LOQ was determined to be 0.1 ng/mL (CV = 18%). The assay was compared to the Beckman Access Thyroglobulin assay (N=37, range 0.1-500 ng/mL). The Spearman correlation coefficient was 0.990 with a slope of 1.28 and intercept of -0.14 by Passing-Bablock regression fit. Comparison with the in-house Tg LC-MS/MS assay (N=129, range 0.5-500 ng/mL) produced a Spearman correlation coefficient of 0.943 with a slope of 1.58 and intercept of -0.69 by Passing-Bablock regression fit.

Conclusion: The Roche Elecsys Tg II shows good analytical performance and provides reliable Tg measurement for the management of thyroid cancer patients.

A-057

Single Cell Analysis of Heterogeneous Circulating Tumor Cell Populations

L. M. Millner[1], K. S. Goudy[2], M. W. Linder[3], R. Valdes Jr[3]. *[1]University of Louisville, Louisville, KY, [2]PGXL Technologies, Louisville, KY, [3]University of Louisville and PGXL Technologies, Louisville, KY*

Introduction: Enumeration of circulating tumor cells (CTCs) in blood is used in breast cancer patients as an independent predictor of outcome. Present methods do not distinguish subtypes and only detect epithelial-type CTCs. This is significant because CTCs experience epithelial to mesenchymal transition (EMT), a process that increases motility, disease progression, and decreases epithelial marker expression. This process may also change the CTC susceptibility to chemotherapeutics and eligibility for treatment. For example, patients with overexpression of HER2 are eligible for treatment with Herceptin. Examining CTCs as a bulk population may mask individual overexpression of markers used for targeting therapy. Here we describe a comprehensive method for characterizing the molecular heterogeneity of CTCs which could play an important role in directing personalized cancer therapeutics.

Objective: To develop a method for identifying single cell heterogeneity within a population of circulating tumor cells.

Methods: As a model, we used the breast cancer cell line MDA-MB-231 (MDA). This cell line was chosen because it has low expression of EpCAM and may represent cells that have experienced EMT. MDA cells are typically negative for EpCAM and HER2 protein expression but do express CD44. Cells were sorted into single cell populations using DEPArray Technology (Silicon Biosystems) and analyzed by single cell RT-PCR for 3 targets (EpCAM, ErbB2 (HER2), and CD44) and a housekeeping gene (ACTB). Using DEPArray technology the mean fluorescent intensity (MFI) of EPCAM, CD44 and HER2 protein expression was measured on 3,656 individual cells and MFI signals greater >1000 were considered positive.

Results: ACTB transcript expression as a positive control was confirmed in each of the eleven cells. EpCAM transcript expression was not detectable in any of the cells. CD44 transcript expression was observed in 10/11 (91%) and ErbB2 (HER2) expression was observed in 4/11 (36%) cells. We then measured the mean fluorescent intensities (MFI) for each of the cell surface target antigens on 3,656 individual cells. The MFI values [mean ± SD (range)] were: EpCAM, 525±91 (369-870); CD44, 3722±1598 (1223-13963), and HER2, 562±154 (389-6312).

Conclusion: The wide range of MFI signaling for HER2 demonstrates a discrete subpopulation of cells expressing high levels of HER2 within a population that on average expresses no or very low levels of HER2. This single cell analysis method may provide identification of a subpopulation of Herceptin-responsive cells within an apparently non-responsive group. Single cell subtyping has the potential to facilitate individually tailored therapies based on each patient's heterogeneous CTC profile.

A-058

Prognostic Biomarker Isocitrate Dehydrogenase-1 Mutations in Patients with Glioblastoma Multiforme

J. Polivka[1], J. Polivka[2], V. Rohan[2], M. Pesta[3], T. Repik[2], O. Topolcan[4]. *[1]Department of Histology and Embryology and Biomedical Centre, Faculty of Medicine in Plzen, Charles University in Prague, Plzen, Czech Republic, [2]Department of Neurology, Faculty of Medicine in Plzen, Charles University in Prague and Faculty Hospital Plzen, Plzen, Czech Republic, [3]Department of Biology, Faculty of Medicine in Plzen, Charles University in Prague, Plzen, Czech Republic, [4]Central Imunoanalytical Laboratory, Faculty Hospital Plzen, Plzen, Czech Republic*

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

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

Results The IDH1 R132H mutation was identified in 20 from 44 GBM tumor samples (45,4%). The majority of mutated tumors were secondary GBMs (16 in 18, 89.9%). Low frequency of IDH1 mutations was observed in primary GBMs (4 in 26, 15.3%). Patients with IDH1 R132H mutation had longer PFS - 136 vs. 51 days ($P<0.021$) as well as OS - 270 vs. 130 days ($P<0.024$).

Conclusion The prognostic value of IDH1 R132H mutation in GBM patients was observed in our study. Patients with this mutation had significantly longer PFS and OS than patients with wild-type IDH1 and suffered more likely from secondary GBMs. The IDH1 mutation status could be used as a strong prognostic factor for patients with GBM and should be further studied in larger patient cohort.

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A-059

New monoclonal antibodies detect all immunoglobulin free light chains in urine samples from over 13,000 patients

J. Campbell, J. Heaney, Y. Wang, M. Cobbold, M. Goodall, T. Plant, M. Drayson. *University of Birmingham, Birmingham, United Kingdom*

A decade or so ago, the first automated assay was launched for the quantitation of serum κ and λ immunoglobulin free light chains (FLC). FLC measurement is now a fundamental procedure in the diagnosis and monitoring of patients with plasma cell dyscrasias including multiple myeloma and related subtypes including light-chain-only, oligosecretory and non-secretory myeloma. Despite these advances, the assay, which uses sheep polyclonal anti-human FLC antibodies, has a number of well-observed limitations. It has been proposed that monoclonal antibodies (mAbs) may overcome these limitations. The development of FLC specific mAbs is difficult because the mAbs must demonstrate specificity for epitopes that are exposed on FLC but hidden on LC bound to whole immunoglobulin. This is complicated by the paucity of constant domain epitopes available; which can be further reduced by polymerisation of FLC, particularly FLC λ, thus reducing the number of potential binding sites. Production of mAbs specific for FLC has been described previously but other groups have either found that their mAbs did not detect FLC from all neoplastic plasma cell clones tested, or, have not tested sufficient clones to be confident that the mAbs would detect the FLC from 100% of neoplastic clones. Hence, the purpose of this study was to prospectively assess the clinical utility of new highly-specific mouse anti-human FLC mAbs on a large number of consecutive patient samples. Anti-κ and anti-λ FLC mAbs were covalently coupled to different polystyrene Xmap® beads and assayed, simultaneously, in a multi-plex format by Luminex® (mAb assay). The mAbs displayed no cross-reactivity to bound LC, the alternate LC type, or other human proteins and had improved sensitivity (<1mg/L) over the gold standard for identifying paraprotein, immunofixation electrophoresis (IFE; approximate sensitivity is 10mgL). The competitive inhibition format gave a broad calibration curve (up to 437.5 mg/L) and prevented anomalous results for samples in antigen excess (i.e. high FLC levels). The mAb assay had no false negatives and identified all monoclonal FLC in 13,090 urine samples tested (22.8% with monoclonal κ and 9.0% with monoclonal λ by IFE), and also detected all samples with polyclonal FLC. In a small cohort of Bence Jones positive samples (n=100), the mAb assay correlated excellently with densitometry, the gold standard for quantitating urine FLC. Importantly this shows that the mAbs are close to the ideal of detecting FLC from all patients and neoplastic plasma cell clones, and may be the first published mAbs with this clinical utility. Given the overall effectiveness of the anti-FLC mAbs, further clinical validation is now warranted on these mAbs in other assay platforms they are incorporated, including Seralite, a rapid (10-minute) and portable FLC test to be used at the point-of-care.

A-060

Development of RT-qPCR Assays for The Detection of Circulating Tumor Cells in Breast Cancer

S. Ahn[1], S. Park[2], H. Wang[3], S. Park[1], S. Kim[4], Y. Kim[1], H. Kim[1], G. Kim[1], J. Kim[1], H. Jin[5], S. Kim[6], H. Lee[1]. *[1]Department of Biomedical Laboratory Science, College of Health Sciences, Yonsei University, Wonju, Korea, Republic of, [2]Department of Clinical Laboratory Science, College of Health and Therapy, Daegu Hanny University, Daegu, Korea, Republic of, [3]M&D, Inc., Wonju Eco Environmental Technology Center, Wonju, Korea, Republic of, [4]Institute for Life Science and Biotechnology, Yonsei University, Seoul, Korea, Republic of, [5]Department of Clinical Laboratory Science, College of Health Sciences, Catholic University of Pusan, Busan, Korea, Republic of, [6]Department of Surgery, College of Medicine, Yonsei University, Seoul, Korea, Republic of*

Background: Cancer cells which become detached from the primary tumors and enter into the systemic circulation are called circulating tumor cells (CTCs). The human epidermal growth factor receptor 2 (*HER2*, also known as *erbB2*) is crucial for treatment of breast cancer patients. HER2 is over-expressed in 20 to 30% in blood samples of breast cancer patients. Especially, HER2 gene's over-expression is associated with a poor clinical outcome. Therefore, the detection of HER2 expressing CTCs in the blood may have important prognostic and therapeutic treatment implications. In this study, apart from using HER2, EpCAM, CK-19, Ki-67, and hTERT were used for detection CTCs in peripheral blood of breast cancer patients. Furthermore, correlation between HER2 and CTC markers mRNA level in the blood were determined.

Methods: Human breast carcinoma cell line SK-BR-3, MCF-7 and MDA-MB-231 were used for the development of the assay and the confirmation of HER2, EpCAM,

CK-19, Ki-67, hTERT and GAPDH expression. A Total of 188 breast cancer patients who include 34 ductal carcinoma in situ (DCIS) patients, 93 stage I patients, 58 stage II patients and 3 stage III patients. A total of 50 healthy donors who did not have a breast cancer were also enrolled for this study. All blood samples were handled for extracting total RNA using TRIzol Reagent (Invitrogen, Carlsbad, California, USA) then the cDNA was synthesized. The mRNA expression levels of HER2, EpCAM, CK-19, Ki-67, and hTERT relative to GAPDH were measured by RT-qPCR TaqMan assay.

Results: Among a total of 188 patients, 39 patients (20.7%) displayed an over-expression of HER2 mRNA, while none of the healthy blood donors over-expressed HER2 mRNA. In 37 out of 39 HER2 positive patients, not only HER2 mRNA, but also at least one other type of marker was overexpressed at the same time. Among the 149 HER2 negative patients, 114 patients (76.5%) were positive at least one other type of marker. The HER2 mRNA levels in blood had a correlation with Ki-67 mRNA level (Pearson r=0.4358, R square=0.2360) and hTERT mRNA level (Pearson r=0.2988, R square=0.0893) in blood. As the breast cancer stage progress, patients who were over-expressed tumor association markers, such as hTERT, Ki-67, and HER2 were tend to increasing. On the other hand, expression of CTC epithelial markers, such as EpCAM and CK-19 not seem to have correlation with stage of cancer.

Conclusion: In conclusion, HER2 expression in the blood occurs concurrently with CTC markers. In this reason, CTC markers could be used for detection CTCs in blood of breast cancer patients. The results from this study seems to suggest that detection of CTCs using CTC markers and HER2 allow for more effective management of and better prognosis for breast cancer.

A-061

Analytical Evaluation of a Newly Developed ELISA for the Detection of Soluble Tumour Necrosis Factor Receptor 2 (sTNFRII) in Sera from Patients with Ovarian Cancer

A. Chacko, P. Ratcliffe, L. Stevenson, J. Lindsay, P. Lowry, R. McConnell, S. FitzGerald. *Randox Laboratories Limited, Crumlin, United Kingdom*

Background: There has been significant interest in discovery of biomarkers and biomarker panels for early detection of precancerous ovarian tumours. Various screening strategies combining serum-based markers with other clinical parameters are being tested in on-going trials. To date, no serum-based markers have been proven to improve diagnostic performance over the standard ovarian cancer marker CA-125, either alone or as part of a biomarker panel. Previous studies have suggested that sTNFRII levels may increase during tumour progression in different cancer types, including ovarian cancer. This study reports the analytical evaluation of a new developed enzyme-linked immunosorbent assay (ELISA) for the specific and sensitive detection of sTNFRII and its applicability to the study of patients with ovarian cancer. Methods: Sheep were immunized with the extracellular domain of the sTNFR II recombinant protein. Lymphocytes were collected and fused with heteromyeloma cells. Hybridomas were screened for immunoreactivity against native human sTNFRII. Hybridomas which showed strong reactivity to native antigen and <1% reactivity to cross reactants were selected for cloning to produce stable monoclonal hybridomas. An optimal antibody pair was identified for development of a sandwich ELISA platform for detection of sTNFRII in human serum. sTNFRII levels were determined in sixty one serum samples (34 from ovarian cancer patients and 27 healthy female controls). Statistical analysis was performed and box plots / Receiver Operating Characteristics (ROC) curves were constructed using GraphPad Prism. Results: The assay detected native sTNFRII across an assay range of 0-16ng/ml. The limit of detection,defined as the analyte concentration corresponding to an absorbance equal to blank mean plus 2xSD (n=20), was 0.324ng/ml. Intra-assay precision data (n=12) showed recovery at 103.6% ±10.4% (10.0% CV) for 2ng/ml, and 86.5% ±5.1% (5.9% CV) for 4ng/ml sTNFRII. Patient sera were run at a dilution of 1 in 5 to best match the expected clinical range for sTNFRII, giving an effective assay range of 1.6-80ng/ml. Median sTNFRII levels were significantly increased in ovarian cancer patients compared to healthy females (22.7ng/ml v 5.3ng/ml, Mann Whitney $p<0.0001$), which gave an area under the curve (AUC) of 0.907 for ovarian cancer versus healthy females. Conclusion: The results indicate that this newly developed ELISA is applicable to the specific detection of sTNFRII in human serum. The significant increase in median sTNFRII levels in serum from ovarian cancer patients, when compared to controls, indicates that this assay may be suitable for further studies into the use of sTNFRII as a marker for cancer diagnosis and monitoring.

A-062

Examination of Thyroglobulin and Thyroglobulin Antibody Testing Processes for an Urban Endocrine Center

S. E. Wheeler, L. Liu, H. Blair, O. Peck Palmer. *University of Pittsburgh, Pittsburgh, PA*

BACKGROUND: The American Society of Cancer estimates in 2014 ~62,980 individuals will be diagnosed with thyroid cancer. Patients and physicians require accurate and timely in-house thyroglobulin results. Specifically, in differentiated thyroid carcinoma (DTC), the most common thyroid cancer, thyroglobulin (Tg) is used to assess disease recurrence in patients who have undergone thyroidectomy. Commercially available Tg immunoassay methods are most common but are susceptible to Tg antibody (TgAb) interference. In most laboratories TgAb is quantified and manufacturer cutoffs are used to categorize a specimen as TgAb negative (Tg analyzed by immunoassay) or TgAb positive (Tg measurement is referred to an alternate methodology). Common methodologies include the Tg radioimmunoassay (RIA) method and the recently available LC/MS/MS methodology. Examination of alternative test options is critical as referring samples to outside laboratories increases result turnaround time and patient costs.

OBJECTIVE: To determine the optimal testing algorithm for a large endocrine center, we examined the correlation between the current in-house immunoassay methodology for Tg measurement with RIA and LC/MS/MS methodologies using patient specimens categorized with low or high TgAb concentrations.

MATERIALS AND METHODS: Excess samples (n=40; -80°C storage) were obtained from outpatient adults as well as 10 healthy volunteer specimens. Patient specimens were divided into 2 groups: 20 TgAb <20 U/L specimens; 20 TgAb >20 U/L specimens. Samples were analyzed for TgAb using a solid phase enzyme labeled chemiluminescent sequential immunometric assay (Siemens Immulite 2000 XPi, Erlangen Germany; manufacturer's cutoff of 20 U/L). Tg was analyzed using a simultaneous one-step immunoenzymatic assay (UniCel™ DxI 800 automated analyzer, Beckman Coulter, CA; all samples), a RIA method (USC Endocrine Laboratories, CA; used only for samples TgAb >20 U/L), and LC/MS/MS methodology (Quest Diagnostics, Nichols Institute, CA; all samples).

RESULTS: Overall high correlation was demonstrated between the DXI800 and LC/MS/MS methodologies (R^2=0.99; slope = 1.309). Specimens with low TgAb (<20 U/L) demonstrated good correlation between the DXI800 and the LC/MS/MS methodologies (R^2=0.99; slope = 1.312). We examined the effects of TgAb interference (TgAb >20 U/L) and found a good correlation between the DxI800 and the LC/MS/MS methodology (R^2 = 0.97; slope = 1.243). However,Tg measurement between the RIA and LC/MS/MS methods was lower (R^2 = 0.82) particularly for specimens with Tg concentrations >13ng/mL (>13 ng/mL; R^2 = 0.67). TgAb interference was reflected in the method comparison of the DxI 800 and RIA (R^2 = 0.76; slope = 1.216) methods. We observed high variability between TgAb methods similar to previous studies.

CONCLUSIONS: The high correlation between LC/MS/MS and DXI800 suggests that both methods may be appropriate for our patient population. However, before LC/MS/MS testing is placed into routine use our findings warrant validation in a larger TgAb defined population.

A-063

The upregulation of ICAM-1 mediated by leptin is Rho/ROCK-dependent and enhances gastric cancer cell migration

C. Wang, Z. Dong, Y. Yang, L. Du, G. Zheng, G. Zheng, W. Li, X. Zhang, Z. Li, L. Wang, J. Li, H. Liu. *Qilu Hospital, Shandong University, Jinan, China*

Background: Gastric cancer (GC) ranks as the second leading cause of cancer-related death in the world. Adipocytes provide fatty acids for rapid tumor growth, and the dysfunction of lipid metabolism can lead to the pathogenesis of human GC. Leptin is an adipokine of the obesity (ob) gene, and our previous study showed that leptin promote GC cell invasion by AKT/MT1-MMP pathway. However, the exact effect and the underlying mechanism of leptin in GC metastasis remain unclear. Intercellular adhesion molecule-1 (ICAM-1) is overexpressed and plays crucial roles in tumor metastasis. This study aimed to characterize the influence of leptin on ICAM-1 expression in GC and elucidate its underlying molecular mechanism.

Methods: Archived paraffin-embedded GC tissues and matched adjacent normal gastric tissues were collected from 84 patients who underwent surgery for primary gastric carcinoma. The expression of leptin and ICAM-1 were detected by immunohistochemistry, and the correlation of two proteins was further analyzed. The

effect of leptin on GC cell (AGS and MKN-45 cells) migration was measured by transwell. The level of ICAM-1 at both mRNA and protein were detected by RT-PCR and western blot after treatment with leptin. Moreover, the cell surface ICAM-1 and sICAM-1 were detected by flow cytometry and ELISA. ICAM-1-siRNA was designed and transiently transfected in GC cells. RhoA GTPase activity was detected using the G-LISA RhoA activation assay kit. Correlations of leptin and ICAM-1 expression with clinicopathologic factors were analyzed by Kruskal-Wallis test or Mann-Whitney U test, as appropriate. Chi-squared test was applied to analyze the correlation of leptin and ICAM-1 respectively. Other data from experiments were analyzed by paired Student's t-test or one-way ANOVA wherever appropriate. $P<0.05$ was statistical significance.

Results: Immunohistochemical analysis revealed that leptin (48/84, 57.1%) and ICAM-1 (54/84, 64.2%) were overexpressed in GC tissues, and they were positively correlated with each other ($P<0.001$), as well as with the clinical stage and lymphatic metastasis. In transwell assay, leptin promoted GC cell (AGS and MKN-45) migration in a time- and dose-dependent manner. Furthermore, leptin induced GC cell migration by upregulating ICAM-1 expression (mRNA: 4.06±0.54-fold for AGS, $P<0.001$; 2.56±0.33-fold for MKN-45, $P=0.005$. Protein: 3.07±0.25-fold for AGS, $P<0.001$; 2.9±0.26-fold for MKN-45, $P=0.003$), and knockdown of ICAM-1 by small interference RNA (siRNA) blocked this process (AGS: 53.9%±3.6%, $P=0.020$; MKN-45: 42.79%±3.78%, $P=0.005$). Notably, the surface expression of ICAM-1 (AGS, $P<0.001$; MKN-45, $P<0.01$), as well as the soluble ICAM-1 (sICAM-1) (AGS, $P<0.05$; MKN-45, $P<0.01$), was also enhanced by leptin. Moreover, leptin increased ICAM-1 expression through Rho/ROCK pathway, which was attenuated by pharmacological inhibition of Rho (C3 transferase) at 0.25 μg/mL (AGS, $P<0.01$; MKN-45, $P<0.01$) or inhibition of its downstream effector kinase Rho-associated protein kinase (ROCK) (Y-27632) at 3.3 μM (AGS, $P<0.01$; MKN-45, $P<0.01$), suggesting an essential role of Rho/ROCK pathway in this process.

Conclusions: Our findings indicate that leptin enhances GC cell migration by increasing ICAM-1 expression through Rho/ROCK pathway, which may provide preliminary experimental clues for the development of new therapies against the metastasis of GC.

A-064

hCG candidate epitopes for improving the measurement of hCG: results from the second ISOBM TD-7 workshop

P. M. Hemken[1], E. Paus[2], C. Sturgeon[3], W. Stewart[4], J. Skinner[1], L. Harwick[1], S. Saldana[1], C. Ramsay[1], K. Rupprecht[1], K. H. Olsen[2], J. M. Bidart[5], U. H. Stenman[6], P. Berger[7]. *[1]Abbott Laboratories, Abbott Park, IL, [2]Oslo University Hospital, Oslo, Norway, [3]Royal Infirmary, Edinburgh, United Kingdom, [4]Ninewells Hospital and Medical School, Dundee, United Kingdom, [5]Institut Gustave-Roussy, Villejuif, France, [6]Helsinki University Central Hospital, Helsinki, Finland, [7]University Innsbruck, Innsbruck, Austria*

The purpose of this collaboration was to determine specificity profiles and epitopes recognized by diagnostically relevant antibodies (Abs) directed against human chorionic gonadotropin (hCG). This provided the basis for improving the measurement of hCG by harmonization of epitopes of the Abs used and to build broad assay specificity consensus for use as a tumor marker, pregnancy and pregnancy related disorders.

Eight companies and research groups submitted 69 Abs directed to hCG and hCG-related variants. Each of these Abs were characterized in detail by the participants of the Second International Workshop (WS) on hCG of the International Society of Oncology and Biomarkers Tissue Differentiation 7 (ISOBM TD-7).

To determine the specificities of the Abs, the First WHO International Reference Reagents for six hCG variants, hCG, hCGn, hCGβ, hCGβn, hCGβcf, and hCGα were used. Seventeen reference monoclonal (m)Abs were used to assign molecular epitope localizations for the ISOBM-mAbs in the WS. This was performed by comparing ISOBM-Ab specificity, sandwich compatibility, mutual inhibition profiles and affinities, to mAbs of known epitope specificities.

The data shows that 48 mAbs recognized hCGβ, 8 hCGα-, and 13 αβ-heterodimer-specific epitopes. Twenty-seven mAbs were of pan hCG specificity. Two of these pan hCG mAbs had very low cross-reactivity with hLH (<0.1 %; epitope β_1), 12 with low hLH cross-reactivity (<1.0 %; epitopes $\beta_{2/4}$), and 13 with high hLH cross-reactivity (>>1 %; epitopes $\beta_{3/5}$). Four mAbs recognized epitopes on hCGβcf-only (e.g., epitopes β_{11} and β_{13}) and six mAbs epitopes on the remote hCGβ-carboxyl-terminal peptide (epitopes β_8 and β_9).

For routine diagnostic measurements, methods are used that either detect hCG-only, hCGβ-only, or hCG together with hCGβ or hCG together with hCGβ and hCGβcf. Sandwich assays that measure hCG plus hCGβ and eventually hCGβcf should recognize the protein backbone of the analytes preferably on an equimolar basis, should not cross-react with hLH and not be susceptible to blunting of signal by nonmeasured variants like hCGβcf. Such assays can be constructed using pairs of mAbs directed against the cystine knot-associated epitope β1 in combination with epitopes β2 or β4 on hCGβ peptide loops1+3 protruding from the central cysteine knot.

In summary, the results of this hCG ISOBM TD-7 WS[1] in combination with those of the First WS[2] enable recommendations to be made regarding epitope combinations to be used for the design of immunoassays for hCG and its variants.

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A-066

High sensitivity detection of residual disease in multiple myeloma using mass spectrometry

J. R. Mills, D. R. Barnidge, D. Murray, J. A. Katzmann, A. Dizpenzieri, D. L. Murray. *Mayo Clinic, Rochester, MN*

Background:Traditionally, detection of an M-protein (monoclonal immunoglobulin) has been used to diagnose and monitor multiple myeloma (MM). As therapies for MM have improved, more sensitive methods have been used to define response: immunofixation electrophoresis (IFX) of serum and urine, normalization of the serum immunoglobulin free light chain (FLC) ratio, and high sensitivity flow cytometry to detect clonal plasma bone marrow cells. It is hoped that these more sensitive approaches will differentiate those patients with minimal residual disease (MRD) versus no residual disease (NRD), the latter which could mean cure. Flow cytometry of plasma cells requires bone marrow aspiration, which is inconvenient and expensive and is potentially limited by sample bias. More sensitive methods to differentiate MRD from NRD using serum would be advantageous.

Objectives:To develop a high sensitivity method to detect residual M-protein secreted from malignant plasma cells as a means to monitor minimal residual disease (MRD) in serum.

Methods:We developed a microLC-ESI-Q-TOF mass spectrometry assay to detect the presence and accurate mass of malignant-specific monoclonal immunoglobulins, a method we termed monoclonal-immunoglobulin-Rapid-Accurate-Mass-Measurement (miRAMM). Briefly, serum immunoglobulins are enriched and then reduced with DTT, and samples are separated using an Eksigent-Ekspert LC system using a Poroshell C3 1x75mm column running at 25μL/min with gradient of aqueous (0.1 % formic acid) to organic (90:10 ACN/IPA) over 24 minutes. The accurate mass of monoclonal light chains is determined by deconvolution of the mass spectra of multiply charged ions across the retention times of immunoglobulin light chains. Serum samples from 14 patients (cohort #1) positive for M-proteins by serum protein electrophoresis (SPEP) and IFX were collected. Neat and diluted serum (diluted 1:1000 in pooled serum from healthy donors) was analyzed by IFX, SPEP, FLC and miRAMM. Cohort #2 included pre-and post-therapy serum from 21 MM patients who had been classified to have achieved stringent complete response (sCR) post; sCR is defined as an undetectable M-protein by SPEP, a negative FLC ratio, a negative IFX, and absence of clonal plasma cells by 4-color flow cytometry from bone marrow. The accurate mass of disease-associated light chains determined by miRAMM pretherapy was used to test for MRD post-therapy in the sCR patients.

Results:For patients in cohort #1 we were able to identify and determine the accurate molecular mass of the monoclonal light chains in all cases (14/14; 100%). To further test the relative sensitivities of each assay, these same patients' samples were subject to 1:1000 dilution into pooled human serum and retested by SPEP, IFX, FLC ratio, and miRAMM. A monoclonal was detected in 0/14, 2/14 (14%), 0/6, and 13/14 (93%), respectively. In addition, of 21 patients in cohort #2 who had been classified to have achieved sCR by conventional means, including 4-color flow cytometry of bone marrow, 67% had detectable residual monoclonal light chains by miRAMM.

Conclusion:This study demonstrates that miRAMM is a more sensitive approach to detect MRD compared with current methods. This method provides additional value in that the accurate mass measurement provides a unique molecular mass identifier of an individuals' malignancy.

A-067

Identification of four molecular subclasses of Luminal Breast Cancer with different likelihood of recurrence and response to Tamoxifen

R. A. Abbud-Antaki, A. Deluca. *Falcon Genomics, Inc., Allison Park, PA*

Background: Genomic studies have revealed four main molecular subtypes of breast cancer with significant heterogeneity within each class. Several genomic assays are now being used in the clinic for prediction of recurrence in breast cancer patients, and determination of which patients could be spared from Chemotherapy. The objective of this study is to develop a reliable clinical diagnostic test for breast cancer patient classification.

Results: We report on a gene expression-based test for classification of breast cancer tumors that identifies additional subsets of Luminal breast cancer with differences in prognosis and response to Tamoxifen therapy. For these studies, we employed Affymetrix HG-U219 GeneAtlas microarrays to examine gene expression profiles from breast cancer tissue (n=17) and normal adjacent tissue (n=6). When we performed Supervised hierarchical clustering using the previously published "intrinsic gene" signature, we could not obtain definite classification for each patient tumor. This has motivated us to come up with a quantitative approach to breast cancer patient classification.

We first examined expression of genes that are commonly used in the pathological evaluation of breast cancer tissue. These were: ESR1, PGR, ERBB2, AR, luminal keratins (KRT 8, 18, 19), basal keratins (KRT 5, 6B, 14), and Cadherins (E-CDH, OB-CDH, and P-CDH). We then classified our patient tissues according to luminal, basal KRTs, E-CDH, and OB-CDH. This gave us 8 different classes with 380 differentially expressed genes. Only 30 out of the 380 genes were found in the "intrinsic" gene list. The genes that were differentially expressed between these classes were not limited to those related to the luminal and basal phenotypes; other differentially expressed gene clusters included proliferation and stromal genes.

We then validated the ability of this signature to classify published breast cancer gene expression datasets (GSE2034, GSE5327, GSE1561, GSE26971, GSE9195, and GSE6532). Supervised Hierarchical Clustering and Gene Set Enrichment Analysis using our 380-gene signature revealed that Luminal cancer patients could be further divided into 4 groups (L1-L4). L1 samples, also known in the literature as Luminal-B, expressed high levels of proliferation genes and showed the highest likelihood of recurrence among untreated patients, but responded well to Tamoxifen treatment. However, the likelihood of recurrence after Tamoxifen for these patients only became similar to other untreated Luminal groups (L3-L4). However, the likelihood of recurrence for L2 patients was similar irrespective of Tamoxifen. L3 patients, on the other hand, showed statistically significant increase in survival after Tamoxifen, but it was not as good as L4 patients. Some patients had additional genetic abnormalities that made them susceptible to relapse. L4 patients had a better prognosis, with significantly improved survival after Tamoxifen.

Conclusions: Our 380-gene classifier is capable of providing comprehensive classification of breast cancer patients. This test could be used in the clinical setting for identification of the major molecular classes of breast cancer and further sub-classification of luminal patients into 4 classes with different rates of recurrence. This will allow for the early identification of patients that would not respond to Tamoxifen therapy.