clearance is a marker of pathogenicity, diagnosis or prognosis in plasma cell dyscrasias requires further study.

1338 POSTER

Identification of potential diagnostic markers in bronchial fluid of patients with non small cell lung cancer (NSCLC)

S. Carrera[1], R. Zalacain Jorge[2], J. Algorta[3], A. Sancho Gutierrez[4], B. Calvo Martinez[5], I. Rubio Etxebarria[4], X. Mielgo Rubio[4], E. Iruarrizaga Ovejas[4], U. Aresti Goiriena[5], G. Lopez Vivanco[4]. *[1]Cruces Hospital, Medical Oncology, Barakaldo-Vizcaya, Spain; [2]Cruces Hospital, Pneumology, Barakaldo-Vizcaya, Spain; [3]Progenika, Biopharma, Zamudio-Vizcaya, Spain; [4]Cruces Hospital, Medical Oncology, Barakaldo-Vizcaya, Spain; [5]Cruces Hospital, Molecular Biology-medical Oncology, Barakaldo-Vizcaya, Spain*

Background: Lung cancer ranks among the most common and most lethal malignancies worldwide. Given the fact that survival of lung cancer patients is very poor, it is logical to speculate that early detection might result in more favourable outcomes for these individuals. New proteomic techniques can identify potential diagnostic and prognostic markers. The aim of this study was to find protein markers in bronchial fluid which could enable early diagnosis in NSCLC.
Materials and Methods: We have included 96 patients with NSCLC diagnosed using bronchoscopy (64 scamous/29 adenocarcinoma/3 others) and 49 consecutive patients with non pathological bronchoscopy. Bronchial fluid was obtained from each patient and potential protein markers were studied. Bronchial fluid was centrifuged and supernatant proteins were analysed using bidimensional electrophoresis with poliacrilamid gel stained with silver nitrate. Gel was scanned and analysed with *Progenesis* PG6220 program, which measures intensity of each spot. Resultant intensities in each group of patients (NSCLC/non pathological bronchoscopy) were compared using T-Student method. We selected as potential markers those spots with a p value of less than 0.05. We calculated "fold change" of each spot as the ratio between mean intensity in NSCLC bronchoscopies samples and non pathological bronchoscopies samples.
Results: We analysed 300 spots in each sample and we found 31 potential markers whose fold-change ranges from 1.49 to 7.41; 15 of the markers were expressed in a higher level in NSCLC samples and the other 16 were expressed in a lower level.
Conclusions: We have identified 31 differential protein markers in bronchial fluid among our patients. These results could lead in an early diagnostic test which must be validated in future studies.

1339 POSTER

Free light chains in patients with renal impairment associated or not with hypergammaglobulinemia

S. Claeyssens[1], F. Le Roy[2], V. Brunel[1], M. Quillard[1], M. Godin[2], A. Lavoinne[1]. *[1]Hôpital Charles NICOLLE, Medical Biochemistry, Rouen, France; [2]Hôpital Charles NICOLLE, Nephrology, Rouen, France*

Introduction: Serum immunoglobulin-free light chain (FLC) assay is a major marker in the evaluation and management of patients with plasma cell dyscrasias. However FLC are known to be metabolized by kidneys and these patients frequently present renal insufficiency. We retrospectively assessed the effect of renal impairment on serum and urinary polyclonal FLC values in patients with or without polyclonal hypergammaglobulinemia.
Methods: K and L FLC concentrations were measured by nephelometry (Freelite ®, The binding site) in serum and 24-h urines from 80 patients [73 (22−91) years]. Patients with monoclonal protein detected by serum and/or urinary protein immunofixation electrophoresis were excluded. Three arbitrarily groups of patients with normal serum immunoglobulins concentrations and FLC K/L ratio (rFLC) were defined with respect to type of proteinuria (Hydragel Proteinuria, Sebia) and to K and L FLC renal clairance ratio (CK/CL) as physiologic (C, n = 11), predominant tubular (T, n = 27) or predominant glomerular (G, n = 31) groups. A fourth group of patients had renal impairement and polyclonal hypergammaglobulinemia (H, n = 11). Results: median (ranges); Mann-Withney test (significance: $P < 0.05$), Spearman correlations (significance: $P < 0.02$).
Results: Throughout C, T and G groups, K and L FLC serum concentrations, urinary excretions and renal clearances were significantly inversely correlated with glomerular filtration (as evaluated by 1/serum creatinine concentration), regardless of the type of proteinuria. Serum rFLC was inversely correlated while urinary rFLC and CK/CL were positively correlated with glomerular filtration. In H as compared to G group, glomerular filtration and CK, CL, CK/CL were similar suggesting similar renal impairment; however, in H as compared to G group, FLC serum concentrations of K [101 (57–344) vs 34 (20–120) mg/L, respectively] and L [60 (42–174) vs 34 (15−78) mg/L, respectively], urinary excretions of K [327 (91–1328) vs 140 (35–537) mg/24 h, respectively] and L [57 (24–371) vs 40 (10–252) respectively] and serum rFLC [1.3 (1.0−2.3) vs 1.0 (0.4−1.7) respectively] were significantly higher.
Conclusions: K and L FLC serum concentrations, urinary excretions and serum rFLC increased with progressive renal impairment, an effect that was reinforced by polyclonal hypergammaglobulinemia. Therefore, interval references for K and L values and serum and urinary rFLC probably should be related to creatinine for the evaluation and management of patients with plasma cell dyscrasias presenting renal insufficiency with or without polyclonal hypergammaglobulinemia.

1340 POSTER

Study of EGFR mutation expression in adenocarcinoma of lung and their implication in the specific treatment – institutional experience

J. Rodriguez Delgado[1], C. Gaspar Martinez[2], A. Zúñiga Cabrera[1], M. López Muñoz[3], M. Navarro Hervás[1], M. Soler Tortosa[3]. *[1]Hospital Universitario de la Ribera, Biologial Diagnosic Area, Alzira, Spain; [2]Hospital Universitario de la Ribera, Medical Oncology, Alzira, Spain; [3]Hospital Universitario de la Ribera, Radiotherapy Oncology, Alzira, Spain*

Background: In the last years, the work groups that are contributing to the study of the treatment of the non-small-cell lung cancer (NSCLC), being one of the emergent lines, the epidermal growth factor receptor (EGFR). Retrospective analyses of the biopsies of patients, mutation of the tyrosine kinase domain of the EGFR confer to the patients a strong sensitivity to gefinitib.
Purpose: Examine retrospectively EGFR mutations at exones 18, 19 and 21 to evaluate the prevalence in a small series of our institution with pulmonary confirming biopsy of adenocarcinoma.
Materials and Methods: We studied 23 patients, of them 7 were females an 16 males, with median age was 58 years (32−76). The stage of patients at diagnosis, according the TNM staging system was: 9 patients (39%) were classified at stage I; 1 (4.5%) at stage II; 7 (30.5%) at stage III; and 6 (26%) at stage IV. Depending on personal background, 20 patients (87%) were smokers and the rest (13%) non-smokers. The study took place on samples peripheral blood and paraffin-embedded tissue. The analysis of gene that codifies the EGFR made by means of genomic obtaining of DNA: 1) from blood-EDTA, 2) from the rich paraffin tissue in tumour. The exones identification 18, 19, and 21 of gene EGFR was made by means of PCR. In each series two controls were used: one positive with genomic DNA of a patient control and one negative (without DNA) to detect possible contaminations. The patients previously were informed about the test that was going away to make, following the effective ethical norms in our country.
Results: Mutations were not detected DNA from peripheral blood of the 23 studied cases. Sixteen of 23 (69%) patients harbored mutations in EGFR gene. 4/23 (17%) presented prognostic therapeutic meaning mutations according to those described before, and were 3 cases of deletion LREA in exon 19 and one mutation L858 of exon 21. These 4 cases corresponded to females.
Conclusions: In our environment, the mutations frequency of EGFR gene in adenocarcinoma pulmonary with therapeutic-clinical meaning is very low and predominantly in female sex.
The systematic study of the EGFR gene mutations may allow the individualization of therapy for patiens with lung adenocarcinoma.

1341 POSTER

Potential diagnostic markers in bronchial fluid of small cell lung cancer (SCLC)

G. Lopez Vivanco[1], S. Sergio Carrera[1], L. Simón[2], A. Gómez Bonilla[1], A. Martinez Bueno[1], D. Ballesteros Quintanilla[1], R. Zalacain Jorge[3], I. Marrodan Ciordia[1], A. Buque Martinez[4], E. Azkona Uribelarrea[1]. *[1]Cruces Hospital, Medical Oncology, Barakaldo-Vizcaya, Spain; [2]Progenika, Biopharma, Zamudio, Spain; [3]Cruces Hospital, Neumology, Barakaldo-Vizcaya, Spain; [4]Cruces Hospital, Molecular Biology-Medical Oncology, Barakaldo-Vizcaya, Spain*

Background: Lung cancer is a major cause of mortality worldwide and overall survival rate has not improved significantly over the past 20 years. Although the incidence of SCLC is declining, it remains a worldwide public health problem. An early diagnosis could improve prognosis and survival among these patients. The aim of this study was to identify protein markers obtained from bronchial fluids of SCLC patients which may differ from non-pathological bronchoscopy samples.
Materials and Methods: We have included 43 patients with SCLC diagnosed using bronchoscopy and 49 consecutive patients with non pathological bronchoscopy. Bronchial fluid was obtained from each patient and potential protein markers were studied. After being centrifuged, supernatant proteins were analysed using bidimensional electrophoresis

with poliacrilamid gel stained with silver nitrate. Gel was scanned and analysed with *Progenesis* PG6220 program, which measures intensity of each spot. Resultant intensities in each group of patients (SCLC/non pathological bronchoscopy) were compared using T-Student method. We selected as potential markers those spots with a *p* value of less than 0.05. We calculated "fold change" of each spot as the ratio between mean intensity in SCLC samples and non pathological samples.
Results: Optimal bidimensional gels of each sample were obtained. Among 300 comparable spots, 10 of them were expressed with a different intensity in both groups of patients; 6 of these potential markers were over expressed in SCLC samples, whereas 4 of them were under expressed. The "fold change" of these 10 spots ranges from 1.5 to 8.67.
Conclusions: Different protein markers can be detected in bronchial fluid obtained from SCLC samples. Significant differences in expression of these biomarkers were detected between SCLC patients and non pathological bronchoscopy patients. The development of an early diagnostic test using these proteins must be validated in future studies.

1342 POSTER
NBL1 and anillin (ANLN) genes expression as diagnostic markers for pancreatic carcinoma

M. Olakowski[1], M. Jarzab[2], T. Tyszkiewicz[3], M. Oczko-Wojciechowska[3], M. Kowalska[3], R. Krol[4], M. Kajor[5], W. Bal[2], E. Nowara[2], P. Lampe[2].
[1]Silesian Medical University, Dept. of Digestive Tract Surgery, Katowice, Poland; [2]Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Dept. of Clinical and Experimental Oncology, Gliwice, Poland; [3]Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Dept. of Nuclear Medicine and Endocrine Oncology, Gliwice, Poland; [4]Silesian Medical University, Dept. of General Vascular and Transplant Surgery, Katowice, Poland; [5]Silesian Medical University, Dept. of Pathomorphology, Katowice, Poland

Diagnostic approach to pancreatic tumors is very often limited by the effectiveness of thin-needle biopsy to confirm the malignancy. Thus, search for novel markers specific for pancreatic cancer is highly substantiated. Based on our microarray study, we have chosen to validate two candidate genes, first indicated by a landmark papers of Iacobuzzio-Donahue et al. and not evaluated further for their association with pancreatic cancer.
The aim of our the study was to verify the utility of gene expression of NBL1 (Neuroblastoma, suppression of tumorigenicity 1) and ANLN (Anillin), as novel molecular markers of pancreatic cancer.
Material and Methods: Initial part of study was based on microarray analysis of 18 pancreatic adenocarcinomas and 16 benign samples (9 chronic pancreatitis specimens and 7 from grossly normal pancreas), by HG-U133 Plus 2.0 oligonucleotide Affymetrix arrays. The obtained dataset was pre-processed using GC-RMA method, gene expression values were compared by parametric t-test with False Discovery Rate estimated by Benjamini-Hochberg method. Validation part of the study was carried out in 66 samples: 31 adenocarcinomas and 35 benign specimens (21 samples of normal pancreas and 14 chronic pancreatitis). Real-time quantitative PCR reaction was performed in all validation set samples on Applied Biosystems SDS 7700 machine with Universal Probe Library fluorescent probes (Roche). We analyzed four reference genes: ATP6V1E1, EIF3S10, HADHA, UBE2D2 and normalized the obtained result to the reference index obtained by geNorm software.
Based on microarray data, most pronounced difference was observed for NBL1 gene, with 34.7-fold increase of expression in cancer. This was confirmed by validation study, where NBL1 gene was 9.5-fold over-expressed in cancer vs normal samples. For ANLN gene a gradual increase in expression from normal samples by chronic pancreatitis to large values in pancreatic cancer was observed (cancer/normal 19.8-fold, cancer/pancreatitis 4.0-fold). For both genes we confirmed the statistically significant differences in gene expression between pancreatic cancer, chronic pancreatitis and normal pancreas ($p < 0.0001$, Kruskall-Wallis ANOVA). In post-hoc inter-group comparisons, both genes differentiated between cancer/normal ($p < 0.000001$) and cancer/pancreatitis ($p < 0.000001$ for ANLN and $p = 0.000001$ for NBL1). By ROC curve analysis we showed that combining both markers gives a significant increase in classification accuracy.
Conclusion: NBL1 and anillin are promising markers for pancreatic carcinoma molecular diagnostics.

Radiotherapy and radiobiology

Oral presentations (Thu, 24 Sep, 09:00–11:00) Radiotherapy and radiobiology

2000 ORAL
Clinical validation of atlas-based auto-contours in the head & neck

P.C. Levendag[1], M. Hoogeman[1], D.N. Teguh[1], P. Voet[1], B. Heijmen[1], X. Han[2], A. Al-Mamgani[1]. *[1]Erasmus MC Daniel den Hoed Cancer Center, Radiation Oncology, Rotterdam, The Netherlands; [2]Elekta, CMS Inc., Best, The Netherlands*

Objective(s): For optimal sparing conditions in the H&N, one requires accurate delineation of target volumes and critical normal tissues. For that purpose a CT-based- atlas of the neck levels I-V and guidelines or critical OAR, were developed. Contouring is tedious, time consuming and suffers from large intra- and inter-observer variability's. A promising new tool is Auto-Contouring (AC) by multiple-subject Atlas-Based Auto-Segmentation (ABAS, CMS, Inc.) of CT-images. Our preliminary results with ABAS, validates the accuracy of the delineation process with or without the help of AC, and we present our analyses the amount of reduction in contouring time that can be realized through ABAS.
Materials/Methods: Eleven N0/N+ patients were selected. In all patients the neck levels I-V (both necks), and 19 OARs were contoured by two staff members; total contouring times were recorded. These reference contours were regarded as the gold standard and used as input for ABAS. In 4 of these patients the generated AC were edited by the 2 staff members and editing times were recorded. Next, for 12 clinically IMRT treated patients, 5 experienced observers edited the generated auto-contours (on average 4 patients per observer). In all cases the neck node levels and the 19 defined OARs were auto-contoured and editing times were recorded. Dice coefficients (0 indicates no overlap, 1 a perfect agreement) were calculated to quantify the similarity to the gold standard of the clinically contoured-, the auto-contoured- and the edited structures. Finally, an expert panel scored all AC contours as well as the edited AC contours regarding their adequacy relative to the Atlas: 0 = poor, 1 = moderate, 2 = good. For AC the following scoring system was used: 0 = poor, 1 = major deviation, editable, 2 = minor deviation, editable, 3 = perfect.
Results: The initial contouring time was 180 minutes per patient on average; editing times approximately 39 minutes. The mean Dice coefficients of the AC contours vs. clinically used delineations were 0.7/0.8/0.8 for the neck node levels, parotids, and submandibular glands, respectively. For the AC contours versus the edited AC contours the Dice were 0.8/0.9/0.8. The expert panel scored 100% of the AC of the neck levels as a minor-deviation-editable or better. The expert panel scored 88% of the edited contours as good, where 83% of the clinically used contours were scored as good.
Conclusions: Multiple-subject ABAS of CT images proved to be a useful novel tool in rapid delineation of normal and target tissues. Although editing of the auto-contours is inevitable (39 min), substantial time reduction was achieved by editing instead of contouring from scratch (180 vs. 39 min.). This is even more relevant since the edited contours were of similar or better quality than the clinical ones.

2001 ORAL
Acute toxicity of curative radiotherapy for intermediate risk localized prostate cancer in the EORTC trial 22991

O. Matzinger[1], F. Duclos[1], A. van den Bergh[2], C. Carrie[3], P. Kitsios[4], S. Villà[5], P. Poortmans[6], S. Sundar[7], L. Collette[8], M. Bolla[9]. *[1]Centre Cordonne D'Oncologie – CHUV, Department of Radio-Oncology, Lausanne, Switzerland; [2]University Medical Center Groningen, Department of Radio-Oncology, Groningen, The Netherlands; [3]Centre Léon Bérard, Department of Radio-Oncology, Lyon, France; [4]Bank of Cyprus Oncology Center, Department of Radio-Oncology, Nicosia, Cyprus; [5]Hospital Germans Trias i Pujol- ICO Badalona, Department of Radio-Oncology, Barcelona, Spain; [6]Dr. Bernard Verbeeten Institute, Department of Radio-Oncology, Tilburg, The Netherlands; [7]Nottingham City Hospital, Medical Oncology Department, Nottingham, United Kingdom; [8]EORTC, Headquarters, Brussels, Belgium; [9]Centre Hospitalier Universitaire de Grenoble, Department of Radiation Oncology, Grenoble, France*

Introduction: EORTC trial 22991 randomly assessed the addition of concomitant and adjuvant short-term hormonal therapy to curative conformal/intensity-modulated radiotherapy (RT) for intermediate risk localized prostate cancer. We report the acute toxicity (assessed weekly during RT) for the organs at risk (genito-urinary (GU) and gastro-intestinal (GI)) in relation to radiation parameters.