888 POSTER

Contributions to the improvement of the quality of the images in our system of computer-aided thermography of contact

M. Mogos, I. Moraru, I. Mogos. *Institute of Oncology, Department of Medical Oncologyy II, Bucharest, Romania*

Purpose: The paper presents results obtained by the authors during the last 10 years in the field of contact thermography (CATE). They demonstrated that their own apparatus and their own computer-aided protocol is more efficient, comparing to the previous, in examinations both of superficial and deep cancers.

Methods: Stronger efficiency of CATE was obtained using: (1) picking up of more data in a stationary position and (2) by displacing the thermal sensors along the skin. Using PC and very precise methodology, the thermal map of the investigated area was obtained. Very important is the fact that our method allowed deep tissue thermal analysis, so any internal or superficial tissues could be investigated.

Results: The original apparatus and method CATE enables a non-invasive exploration of the functionality of the whole body, allows many re-examinations, with the lowest costs. In fact, aspects of energetic and metabolic processes could be stored and compared at different time intervals, both for normal and pathological conditions. The CATE system seems to be a real challenge in exploring molecular level of cellular phenomena. The presented apparatus and method could be used for: detection, screening, diagnose, treatment and post-therapeutical follow-up. Further improvements were done: the mathematical processing of thermogenesis values, for obtaining (a) tomo-thermography and (b) phono-thermography.

Conclusions: We appreciate that the above presented apparatus and method could represent a very useful investigating tool, particularly in the oncological field, but also in some clinical and experimental investigations.

889 POSTER

Detection of p53 gene alterations in saliva and brush cytological specimens from oral carcinoma risk patients

M. Martínez de Pancorbo[1], M. López[1], N. Cuevas[1], M. Anzola[1], J. Aguirre[2], J. Videgain[3], J. Aguirregaviria[3], A. Castro[4]. *[1]Univesity of the Basque Country, Z. and Cellular Dynamics, Vitoria, Spain; [2]Univesity of the Basque Country, Estomatology, Lejona, Spain; [3]Oncology Institute, Otolaringology and Maxilofacial Surgery, San Sebastian, Spain; [4]Datagene, Spain*

Purpose: In oral cavity the epithelial cells are frequently removing and exfoliated cells might be detected in saliva and brush specimens. Recurrence and multifocal nature are the most important characteristics of oral cancer and so the identification of genetic alterations, like p53 mutations, in saliva of high-risk patients suggests possibilities for future oral cancer non-invasive screening technique.

Methods: We analyzed two groups of patients at risk of developing a new or recurrent oral carcinoma; one group had a leukoplakia for the first time (20 individues) and the other had a leukoplakia and previously one or two oral carcinomas (20 individues). The samples collected from each patient were: saliva, hair with root and a brush swabbed over the leukoplakia lesions. DNA was extracted from the cells of these samples and exons 4-8 of p53 gene were amplified. The PCR products were analyzed for mutations by SSCP and the bands having the highest probability to contain mutated alleles were carefully cut and the DNA fragments recovered from polyacrilamide gels to be sequenced with the aim of confirming and localizing the mutation.

Results: We identified p53 mutations in saliva and brush citologies from patients at risk of developing an oral carcinoma. 11 of 40 patients evaluated have one or two mutations in p53 gene; 7 of 20 patients with previous carcinoma showed mutations which were present both in saliva and brush. P53 mutations were observed too in 4 of 20 patients without previous carcinoma but only were present in brush cytologies. The mutations detected in saliva and brush correspond to the first group of patients and two of these patients have two mutations in exons 5 and 8 of p53 gene. In the patients with risk of the primary oral carcinoma the mutations were detected only in the brush sample. The mutations are found majority in exons 5 and 8, only one in exon 7 and a polymorphism in exon 6.

Conclusions: These results suggest that inactivation of p53 can be detected using oral cytologies specimens of patients with leukoplakias before malignant lesions are clinically apparent. The presence of p53 mutation in saliva would suggest recurrence. This technique is non -invasiveness, painless, rapidity and may be useful for the follow-up of these patients because of the risk of developed an oral cancer or a recurrence. In conclusion, this technique introduces new possibilities of analysis of tumor-specific molecular markers.

This work was supported by grants from the University of the Basque Country (UPV I02-EA134/96), UPV I02.I02-TB 128/97; the Basque Government (PI-97/27) and FIS project/Basque Government Department of Health (99/09409)

890 POSTER

Initial report of the HER2000 study: a multinational study of her2 status of breast cancer using immunohistochemistry (IHC; Herceptest)

G. Acosta, J. Askaa, M. Bilous, R. Kodet, A. Moreno, G. Oberhuber, J. Olofson, T. Sauer, G. Tomasic. *On behalf of the HER2000 Study Group*

Background: HER2 is an important prognostic and predictive marker in breast cancer, with a HER2-positive status being indicative of aggressive disease, decreased survival and eligibility for the HER2-specific monoclonal antibody Herceptin. Furthermore, it is widely accepted that routine testing for HER2 status is essential for optimal breast cancer management. Until now, no study has examined HER2 status on a global scale. Therefore, we used IHC to obtain epidemiological data on HER2 status in 10 countries worldwide.

Methods: Formaldehyde-fixed, paraffin-embedded sections (3-5 μm) of samples from primary breast tumours or metastases were retrospectively or prospectively tested for HER2 status using the standardised HercepTest IHC kit. All samples were scored 0, 1+, 2+ or 3+ according to standard criteria outlined in the package insert for HercepTest. Some samples were also stained by IHC using other antibodies; these tests were assessed by individual laboratories as negative or positive.

Results: Data are currently available for the initial 4,476 of 10,500 samples tested; 98% were from primary tumours and 90% were fixed in neutral-buffered formalin. Mean patient age was 59.2 years. By the HercepTest, HER2 status was scored as 0 in 46% of samples; 1+ in 26%; 2+ in 14%; and 3+ in 14%. Subsets of these sections were tested by non-standardized IHC staining procedures using a polyclonal anti-HER2 antibody (A0485) on 814 specimens and the mouse anti-HER2 monoclonal antibody CB11 on 1077 specimens. These tests produced higher positivity rates than the HercepTest: 39-45% and 21-53%, respectively. Interestingly, 91% (269/296) of HercepTest 3+ samples for which alternative IHC test results were available were positive on these tests; 83.5% (1324/1584) of HercepTest 0 or 1+ samples were negative on alternative tests. Variation in scores between countries and centres was noted for all tests, except for the HercepTest in Germany.

Conclusions: The overall rates of HER2 positivity obtained using the HercepTest in this study are consistent with those observed in smaller studies. The reproducibility of HercepTest results between centres in Germany may reflect the existence of an established quality assurance programme. These results indicate the importance of HER2 test validation, strict adherence to test protocols and quality control programmes to ensure that HER2 status is determined correctly and the appropriate patients are selected for Herceptin therapy.

891 POSTER

Molecular imaging paradigms and cancer therapy

E.O. Aboagye. *CRC PET Oncology Group; Dept. of Cancer Medicine, Imperial College, Hammersmith Campus, Du Cane Road, London W12 0NN, UK*

Imaging paradigms can be used to enhance the scientific impact of cancer trials. This lecture will focus on the development of imaging paradigms to measure hypoxia, proliferation, angiogenesis, gene expression and enzyme function.

Hypoxia occurs to a variable extent in tumours and is an important determinant of therapeutic response and survival. We have developed SR 4554 as a magnetic resonance spectroscopy (MRS) and positron emission tomography (PET) compatible probe for the measurement of hypoxia. The design features of SR 4554 were consistent with its *in vivo* pharmacokinetics, enzymology of bioreduction, subcellular distribution in spheroids and tumour retention. Differential retention was demonstrated in tumours with different radiobiological hypoxic fraction and following modulation by carbogen and hydralazine. Based on its interesting properties, SR 4554 has been selected for clinical development and is now in Phase 1 trials.

There is the need to develop new assays, which can be used to evaluate novel mechanism-based cytostatic agents in patients. We are developing 2-[^{11}C]thymidine and 2'-[^{18}F]fluorothymidine for measuring antiproliferative

activity by PET. Proof that these probes can measure the inhibition of proliferation in the absence of tumour shrinkage has been provided for trichostatin-A in HT29 tumour bearing mice. The relationship between inhibition of proliferation and specific effects of the drug including histone H4 hyperacetylation has been studied. Clinical validation of 2-[^{11}C]thymidine has also been performed. The retention of 2-[^{11}C]thymidine was shown to correlate with MIB 1 index in gastrointestinal cancers of patients.

Angiogenesis, the development of new blood vessels from preexisting host vessels is mediated by growth factors, the most important of which is vascular endothelial growth factor (VEGF). A novel PET imaging agent for VEGF, [^{124}I]anti-VG76e, has been developed. [^{124}I]anti-VG76e is immunoreactive and has been shown to differentially label human xenografts that express different levels of VEGF. Imaging studies demonstrated maximal localisation of [^{124}I]anti-VG76e at 24 h post injection. In addition to angiogenesis, evaluation of the magnitude, spatial distribution and time course of gene expression can enhance the scientific impact of gene therapy trials. We have employed a marker gene-PET marker substrate paradigm to monitor gene expression *in vivo*. Promoters of interest have been used to drive marker genes (HSVI-thymidine kinase or sodium iodide symporter). Gene expression is determined indirectly by imaging the retention of PET probes, which are substrates for the protein product of the marker gene.

Dihydropyrimidine dehydrogenase (DPD) is the proximal and rate limiting enzyme in the catabolism of 5-fluorouracil. Large variations in enzyme expression occur and have been shown to influence the pharmacodynamics of 5-fluorouracil. Eniluracil is a mechanism-based inactivator of DPD. Proof of mechanism of action of eniluracil in patients was provided by studying the *in vivo* pharmacokinetics of 5-[^{18}F]fluorouracil (5-[^{18}F]FU) by PET. The rapid conversion of 5-[^{18}F]FU by normal liver (the organ with the highest DPD activity) to [^{18}F]fluoro-β-alanine, as well as the hepatobiliary excretion of [^{18}F]fluoro-β-alanine bile conjugates were inhibited by eniluracil. In the tumours of these patients, the reduction in catabolism led to a significant increase in tumour 5-[^{18}F]FU + anabolite levels.

892 POSTER

The role of FDG whole body positron emission tomography in metastatic carcinoma of unknown primary site

M. Osborne, A. Makris, P.J. Hoskin, J. Lowe, J. Emmott, W.L. Wong.
Mount Vernon Hospital, Northwood, UK

Aim: 5-6% of new cancer cases present as cancer of unknown primary site. They represent a heterogeneous group of tumours, often requiring extensive investigation. The subgroup of patients presenting with extracervical metastases tend to have a worse prognosis and whole body imaging with a non-invasive modality in this group is appealing. The aim of this study was to evaluate the role of FDG PET in establishing a primary tumour in this group and assess its influence on their management.

Patients and Methods: 25 patients (13 male, 12 female) mean age 58 years (range 35-86), with histologically (17) or cytologically (8) confirmed metastatic carcinoma were imaged with whole body PET following conventional work up which failed to reveal the primary origin. Sites of presenting metastases were liver (8 patients), bone (5), nodes (4), ascites (2), and mediastinum, scalp, brain, pelvis, chest wall, abdominal wall (1 each). Investigations prior to PET scanning incuded CT scanning in all cases and mammography, endoscopy, MRI, serum tumour markers and immunohistochemistry according to clinical suspicion of primary tumour site. Whole body scan was performed at one hour post injection of 350MBq of FDG.

Results: In all cases the known sites of metastases were confirmed on PET and in 68% of patients other metastatic sites were also identified. 14/25 (56%) PET scans suggested a possible primary site - lung (4), pancreas (3), breast (1), renal (1), oesophagus (1), caecum (1), colon (2), stomach (1). Of these, 1 primary was subsequently confirmed. In one case a primary not visible on the original PET scan was subsequently identified and confirmed on biopsy in the pancreas. 9/25 (36%) PET scans had some influence on management- 8/25 (32%) supported the planned treatment, but only 1/25 (4%) actually altered planned treatment.

Conclusion: In this group of patients with metastatic carcinoma of unknown primary origin PET identified the known metastases in all cases. Although a possible primary was identified in 56%, this could not be confirmed in the majority of cases. The influence on patient management was limited.

893 POSTER

The value of positron emission tomography (PET) for individual therapeutic management in localised cancer of unknown primary (CUP)

D. Rades[1], I. Wildfang[1], G. Kuehnel[2], A.R. Boerner[2], W. Knapp[2], J.H. Karstens[1]. [1]*Dept. of Radiation Oncology and Dept. of Nuclear Medicine [2], Hannover Medical University, Hannover, Germany*

Background: 2-4% of cancer patients present with CUP syndrome. Median survival for localised disease is 20, for disseminated disease 7 months. For localised disease individual therapeutic strategies become more important, as an option for curative treatment is more likely. After conservative diagnostic procedures including MRI and endoscopy the primary is detected in less than 25%. The diagnostic value of PET and its influence on therapeutic strategies were evaluated prospectively.

Patients and methods: From 05/98 to 10/00 a total of 42 patients, 30 female and 12 male, with localised CUP were investigated. Median age was 60 (42-77) years. Presenting site was lymph node metastasis in 34 patients (cervical 25, axillary 5, inguinal 2, mediastinal 1, paraaortal 1), and visceral metastasis in 8 patients (bone 4, liver 2, pleura 1, CNS 1). Distribution of histologies was squamous cell carcinoma 24, adeno carcinoma 10, anaplastic carcinoma 7, and small cell carcinoma 1. After a median of 7 (3-11) diagnostic procedures without detection of primary, but evidence of localised disease, PET was performed with Fluorine-18-fluorodeoxyglucose.

Results: In 26/42 patients (62%) a primary was suggested by PET and later on confirmed (histologically) in 18 patients (43%): carcinoma of the lung 5, tonsil 2, parotis 2, oral cavity 1, hypopharynx 1, larynx 1, breast 1, liver 1, ovary 1, vagina 1, urethra 1, and anus 1. In 5 of these 18 patients beyond localised disease, additional dissemination, not detected by previous diagnostic measures, was diagnosed by PET. Overall, dissemination was detected only by PET in 16/42 patients (38%). In 29/42 patients (69%) the PET result had major influence on selection of definitive palliative or curative treatment (mostly as radiochemotherapy or radiotherapy alone), in 13 patients by detection of the primary, in 11 patients by detection of dissemination, and in 5 patients by detection of both primary and dissemination. After a median follow up of 11 (3-33) months, 1-year-OAS was 77% (17/22): 100% (14/14) for localised and 38% (3/8) for disseminated disease ($p = 0.012$). 1-year-PFS was 60% (15/25) for the whole series: 93% (13/14) for localised and 18% (2/11) for disseminated disease ($p = 0.009$).

Conclusion: In patients with CUP the PET result has major impact on detection of the primary as well as of disseminated disease. Furthermore, it has also relevant consequences on the individual therapeutic management.

894 POSTER

Phase II of cisplatin (CDDP), etoposide (VP16) and gemcitabine (G) in cancer of unknown primary (CUP)

C. Balaña, M. Margelí, J. Manzano, T. Moran, A. Font, A. Abad, R. Rosell.
Hospital Universitari 'Germans Trias i Pujol', Medical Oncology, Badalona/Barcelona, Spain

Purpose: New agents are beeing incorporated to the treatment of CUP. Addition of G to VP16 & CDDP could cover pancreatic and lung cancer which are the commonest origin of this disease. A combination of the 3 drugs was performed in order to study tolerability, and activity measured as the response rate and overall survival.

Methods: All multiple-organ CUP patients (p), with normal renal and liver function were treated with CDDP 70mg/m2 d1, plus VP16: 70mg/m2d 1 & 2 and G 700mg/m2 d 1 & 8, every 21 d. Assessment of toxicity on each cycle (c).Evaluation of response after 3c. G was administered on d 8 if WBC*1500x109 and platelets 100.00x109. G-CSF was administered in subsequent cycles in case of a neutropenic fever.

Results: 18p (15M/3F), Median age 64.5 (33-74y), 14 adenoc (ac), 3 carcinoma (c), 1 squamous cell c, 11p(61.4)*3 invaded sites, K I*70: 90%, 2p brain metastasis. 76 c, 72c evaluable for toxicity, 16p evaluable for response. Response rate was: CR 2 (12.5%) PR 5 (31.3%) SD 4 (25%) PD 5 (31.3%). (ORR: 43.8%). Principal toxicity was mielosuppression. Nadir was on day 15: leucocytes GIII-IV: 18%c, granulocytes 47%c, platelets 11%c. 6(37%)p had a neutropenic fever and needed G-CSF to continue treatment.

Conclusions:CDDP+VP16+Gemcitabine is an active combination in UPC, principal toxicity is mielosuppresion. More data will be shown, as the study is ongoing.