Poster presentations (Mon, 24 Sep, 14:00−17:00)
Paediatric oncology

1408 POSTER

Identification of bipotential cells in both neuroblastic primary tumours and neuroblastoma bone marrow metastasis

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Background: Neuroblastic (NBT) tumors are derived from neural crest stem cells. Double staining cells by neurofilament and calcyclin have recently been proposed as NBT tumor precursor cells. However, in our hands, neurofilaments are expressed in both glial and neuroblastic cells. Instead, we found specific neuroblastic and glial markers in the GD2 membrane staining and nuclear calcyclin immunostaining, respectively. In this study, we searched for GD2/calcyclin coexpressing cells in primary NBT and bone marrow metastasis.
Methods: Immunofluorescence for membrane GD2 (neuroblastic lineage) and nuclear calcyclin (glial lineage) was used independently and simultaneously looking for GD2/calcyclin double stained (bipotential) cells. Fresh frozen sections (n = 11) and bone marrow metastasis specimens (n = 5) were investigated. Endothelial cells were identified by CD34 immunostaining.
Results: GD2 staining was detected in all neuroblastic cells. Calcyclin was detected in the stromal-glial bundles and endothelial cells. 8 of the 11 NBT were evaluated for double staining and 3 different populations were identified: GD2+/calcyclin− neuroblastic cells, GD2-/calcyclin+ Schwannian-like cells and some GD2+/calcyclin+ population, which included neuroblastic-like cells but also some of cells within the stromal bundles. The double staining neuroblastic subpopulation did not form clusters and was surrounded by GD2+/calcyclin− neuroblasts.
All metastatic bone marrow specimens analyzed showed double stained cells in the neuroblastic aggregates. Most cells in such aggregates were GD2+/calcyclin− and only few double staining cells were present.
Conclusions: The presence of neuroblastic cells which coexpress glial and neuronal lineage markers in neuroblastic primary tumors and metastasis shows their bipotential capacity, but it remains unclear whether these cells are undifferentiated neuroblasts giving rise to sustentacular cells or, otherwise, they are multipotential cells maintaining the malignancy of the tumor.

1409 POSTER

Differential expressed genes in favourable versus unfavourable neuroblastoma tumors

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Neuroblastoma (NB), a childhood tumor originating from neural crest cells in the sympathetic nervous system, has a complex biological heterogeneity depending on clinical stage and age at diagnosis. In order to screen for genes involved in tumour development, a global micro array expression analysis was performed on six NB tumours (three favourable and three unfavourable). Data indicated that the expression levels of several important players in the noradrenalin biosynthesis pathway were significantly lower in unfavourable NB tumours compared to favourable. The 92 most significant genes with a fold change above 2.0 between groups were picked out for verification with real-time PCR (with TaqMan Low Density Array cards, Applied Biosystems) on tumours included in the micro array study. Thirteen additional tumours were also analyzed by real-time PCR in order to explore if the expression pattern is applicable to a larger group. The preliminary results show that transcripts encoded by solute carrier family 6 (SLC6A2), transcription factor AP-2 beta (TFAP2B), and chromosome 5 open reading frame 13 (C5ORF13) all show a distinct down-regulated pattern in unfavourable tumours versus favourable. The protein encoded by SLC6A2 is an important mediator in the noradrenalin biosynthesis pathway. Both TFAP2B and C5ORF13 (also known as P311) are known to induce the expression levels of cell-cycle regulator P21. Also, TFAP2B has been shown to regulate expression of genes required for development of tissues of ectodermal origin, such as neural crest. These findings insist us to further explore these genes and their involvement in neuroblastoma development and progression.

1410 POSTER

Frequent impact of ^{18}F-fluorodeoxyglucose positron emission tomography on the staging of children and adolescents with alveolar rhabdomyosarcoma

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Background: Alveolar rhabdomyosarcoma (ARMS) is an aggressive soft tissue tumour with a characteristic translocation involving chromosomes 2 and 13. It is usually found in adolescents, and typically arises in large muscles of the trunk and extremities. Accurate staging is important, in order to reserve the combined modality treatment to those children who might benefit from it. The most difficult patients to treat are those with metastases, they have a five-year survival rate <30%. FDG-PET is a new imaging method; its diagnostic criteria are based on the metabolic activity of tumour cells. In this study we assess the usefulness of ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography (PET) in the detection of metastatic disease in children and adolescents with ARMS.
Materials and Methods: Over a period of 5 years twelve patients with ARMS (median age 15 years, range 8.7−18.5 years) were prospectively recruited into the study. PET findings were correlated with standard staging including CT, ultrasound, bone scan and bone marrow examination. Discordant findings were verified by MRI. No patients presented with brain or pulmonary metastases.
Results: One third (4/12) of staging PET scans was concordant with conventional staging. PET was found to be more sensitive for detecting of nodal, skeletal and soft tissue involvement. In eight patients PET revealed 11 additional ARMS manifestations (3 distant lymph nodes, 5 multifocal bone lesions, and 3 soft tissue infiltrates) and correctly upstaged 5 of 12 children (42%). No false-positive results were observed. Sensitivity for PET and standard staging methods was 100% and 44%, specificity 100% and 100%, and accuracy 100% and 58%, respectively. Clinical management was changed in 42% of patients as a result of FDG-PET findings.
Conclusions: Our results showed that whole-body FDG-PET might improve and simplify the current staging procedure in ARMS. PET should be recommended as a screening method prior to other conventional used imaging modalities to plan a rational staging protocol. Large multicentric prospective studies are necessary to verify this conclusion.Supported by grant MZ0 CR 64203

1411 POSTER

Impact of FDG-PET for staging of pediatric solid tumours: comparison with conventional imaging modalities

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Background: Pediatric solid tumors are sometimes difficult to visualize correctly with conventional imaging such as computed tomography (CT) or magnetic resonance imaging (MRI). Accurate initial assessment of the extent of disease and precise evaluation of the effect of treatment are critical to deliver appropriate therapy. Metabolic imaging using ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) has become a widely used test for the staging of many malignancies in adults, but there is scarce information with this imaging modality in patients with pediatric malignancies. With these considerations in mind, this study was undertaken to ascertain the value of FDG-PET/CT in the staging of patients of pediatric solid tumors in comparing with the results of CT and MRI.
Method: Fifty-three patients with pediatric solid tumors (rhabdomyosarcoma in 16, Ewing's sarcoma in 11, osteosarcoma in 10, neuroblastoma in 8, germ cell tumor in 5, synovial sarcoma in 2, and Wilms tumor in one) had an FDG-PET/CT during staging or restaging evaluation. FDG-PET/CT scans were acquired with a PET/CT device (Aquiduo; Toshiba Medical Systems, Tokyo Japan) 60 min after tracer injection. The FDG-PET/CT was considered positive if uptake greater than the background activity was noted and could not be explained by normal physiology.
Results: One hundred and fifteen sites were evaluated. Fifty-two patients had positive PET scans at the primary sites. Mean standardized uptake values of each type of tumor at diagnosis were 6.2 in rhabdomyosarcoma,

9.0 in Ewing's sarcoma, 6.8 in osteosarcoma, 3.5 in neuroblastoma, 3.4 in germ cell tumor, 3.6 in synovial sarcoma. The sensitivity, specificity, negative predictive value and positive predictive value of FDG-PET/CT staging were 92%, 80%, 50% and 99%, respectively. The sensitivity and specificity of conventional imaging were 91% and 66%. There were four false-negative cases on FDG-PET/CT: bone metastasis of rhabdomyosarcoma, bone metastasis of neuroblastoma, Ewing's sarcoma at cranial bone, and rhabdomyosarcoma at lower leg. The reason of false negative was mainly due to the small size of the tumors. FDG-PET/CT was more accurate than conventional imaging regarding staging of patients with pediatric solid tumors.
Conclusions: The FDG-PET/CT was found to be a useful method with staging and restaging of pediatric solid tumors. It was especially useful to detect multiple disseminated metastases.

1412 POSTER

Ovarian tissue cryopreservation for girls and adolescents with childhood cancer

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Background: Impairment of ovarian function and loss of fertility are long-term adverse effects of cancer treatment and related to the use of high-dose alkylators or abdominal irradiation. For females, ovarian tissue cryopreservation (OTC) is currently the only available means of potentially preserving gonadal function and fertility.
Aims: To report our experience with OTC for female patients with childhood cancer.
Patients and Methods: From November 2000 OTC has been offered to patients before high risk of ovarian failure cytotoxic treatments. The patient and both parents were informed of the risks of the planned treatment for subsequent fertility, the ovarian tissue preservation procedure and the experimental nature of OTC, before informed consent was obtained. The project was approved by the institutional review board. Ovarian tissue harvesting was programmed to take place, if possible, immediately before the sterilizing treatment. Ovarian tissue was collected by means of laparoscopy with three incision points. The whole ovary was excised and the cortical fragmented and cryopreserved. One sample of ovarian cortex was randomly selected for histological analysis.
Results: 23 patients underwent OTC. Diagnoses were Hodgkin's lymphoma (n = 8), Ewing's sarcoma (n = 7), Osteosarcoma (n = 5), high grade Astrocytoma (n = 1), Limphoblastic lymphoma (n = 1), and extrarenal Rhabdoid tumor (n = 1). Cytotoxic therapies consisted of autologous bone marrow transplantation (n = 3), high dosages of alkylating agents (n = 18), and pelvic radiotherapy (n = 2). Mean age at OTC was 14 years (range 10 to 18). For 10 (43%) patients, OTC was performed after chemotherapy onset, because of disease severity (n = 4), relapse (n = 3), administrative or parental decision to delay (n = 3). No surgical complications occurred, except one minor surgical wound infection. The right ovary was usually preserved. In all cases histological examination of the non-preserved fragment was negative for tumor. Three patients have died from the disease (13%).
Conclusions: OTC is feasible for pediatric patients before aggressive chemotherapy and/or radiotherapy treatment protocols. Our experience suggests that it can be systematically offered to all female patients including prepuberal girls.

1413 POSTER

Prognostic influence of minimal residual disease detected by flow cytometry and peripheral blood stem cell transplantation by CD34+ selection in childhood advanced neuroblastoma

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Background: To determine whether neuroblastoma (NB) minimal residual disease (MRD) by flow cytometry (flow) in bone marrow (BM) could predict prognosis and whether tumor cell purging by CD34+ cell selection will impact on disease-free survival.
Methods: NB MRD in BM was evaluated by flow with CD45-FITC-/CD81-PE+/CD56-PECy5+ monoclonal antibodies cocktail. Peripheral blood stem cell (PBSC) was enriched via positive CD34+ cell selection by magnetic-activated cell separation system (MACS).
Results: In 31 patients with CD45-/CD81+/CD56+ cells by flow at diagnosis, eleven of them became negative after average 4 courses of chemotherapy. All of those 11 patients remained alive without evidence of disease. In twenty patients with positive MRD, thirteen of them relapsed and 1 patient died from disease (mean 25.8 months). There was with a significant difference between these two groups. MRD in BM was tested before PBSCT transplantation (PBSCT) for 19 NB patients. Fourteen was negative, four of them relapsed and 10 patients remained alive without evidence of disease. Another 5 patients with positive MRD, all of them relapsed (mean 17 months after PBSCT) with a significant difference between these two groups. Fourteen of 19 PBSC were purged with CD34+ selection procedure. Six of 14 relapsed (mean 18.43 months after PBSCT). Five patients did not purged for CD34+ selection, and 3 of them relapsed with no significant difference between these two groups.
Conclusions: Positive MRD in BM after average 4 courses of chemotherapy and before PBSCT is an unfavorable factor for stage IV NB. CD34+ selection purging for PBSCT may not improve the prognosis for children with neuroblastoma in advanced stage.

1414 POSTER

The relationship between nutritional status and IGF-I and IGFBP-3 in patients with childhood solid tumours

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Purpose: This study was designed to investigate the relationship between nutritional status and serum IGF-I and IGFBP-3 in children with solid tumors including lymphoma without brain tumor.
Methods: Between April 2003 and April 2006, 61 patients with newly diagnosed solid tumors (mean age 8.23±4.93 years) and a control group of 60 healthy children (mean age 8.27±5.12 years) were evaluated in means of anthropometric measurements [height, body weight, weight for height (WFH), mid upper arm circumference (MUAC), triceps skin fold thickness (TSFT), mid-arm muscle circumference (MAMC), body mass index (BMI)], biochemical parameters and serum levels of IGF-I and IGFBP-3. MAMC was calculated from MUAC and TSFT, where MAMC = MUAC − [3.14×TSFT(cm)]. Criteria for malnutrition are as follows; MUAC, TSFT, MAMC and BMI < 5%. A positivity of at least 2 of these criteria was accepted as malnutrition. Patients were divided into two different groups according to disease stages. Group I consisted of Stage I and Stage II patients, Group II consisted of Stage III and Stage IV patients.
Results: WFH and BMI of the patients were not significantly different than the control group ($p > 0.05$) but MUAC and TSFT of the patients were found to be lower than that of control group ($p < 0.05$). Measurements of TSFT, MUAC, MAMC and IGF-I levels were lower in Stage III and Stage IV patients than in patients with Stage I and Stage II ($p < 0.05$). The total malnutrition rate was found to be 31.1%. The IGF-I levels were significantly lower in the patient group than in the control group ($p < 0.001$). The lowest IGF-I value was found in cases with malnutrition. The IGF-I levels were correlated with TSFT ($r = 0.71$, $p < 0.001$), MUAC ($r = 0.590$, $p < 0.001$), and MAMC ($r = 0.41$, $p < 0.001$).
Conclusion: We concluded that in children with solid tumors besides TSFT, MUAC, MAMC measurements IGF-I measurements is of recognizable value for diagnosis of malnutrition.

1415 POSTER

Gonadal function and puberty assessment in pediatric survivors of a childhood cancer

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Background: Longer survival of children with cancer implies growing concern for late effects. Aim: To assess puberty and gonadal function in pediatric survivors, identifying risk factors for gonadal impairment and defining gonadal markers useful in pediatric ages.
Material and Methods: Childhood cancer survivors <19 years were prospectively evaluated and compared with a control group of healthy children. Type of cancer and treatment, pubertal development, basal FSH, LH, testosterone, estradiol and inhibin B were analysed. Adolescent boys had a seminogram done, and pubertal girls a pelvic ultrasound. Statistical analysis: Hormonal serum concentrations between Tanner stages were compared with Kruskal-Wallis test. Hormonal concentrations for each Tanner stage were compared between the study and control group by the Mann-Whitney U test. Student t test compared profile variables, and covariance analysis (age as covariable). Critical hormones' concentrations were calculates as the interquartile range for each hormone/pubertal stage/sex × 1.5. Variables associated with gonadal insufficiency were evaluated with Chi-square and with a logistic regression