pts aged 15−19 with thyroid cancer. Malignant melanoma occurred in 2% (5/248) adolescents. Low incidence of malignant melanoma in our group of pts does not correspond with SEER data presenting 8% pts aged 15−19 with malignant melanoma. In our opinion, such discrepancy exists due to inconsistent referral of some pts in adult oncology and endocrinology departments.
Carcinomas represent 8.3% of all solid tumours and 6.4% of all cancer in total of 248 pts aged 15−19 years. Epithelial cancer was 2x more frequent in adolescents than in pts under 15 years of age. Low incidence of thyroid cancer and malignant melanoma in our group of pts may be explained by treatment in adult oncology centers in part of adolescents.
MZ0FNM2005

4122 POSTER

Application of NKX2, STEAP1 and CCND1 Genes Expression for Bone Marrow Involvement Detection in Patients With Ewing Family Tumours

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Background: Ewing family tumours in children are highly aggressive diseases characterized by frequent distant metastases. The most common metastatic sites are lungs, bones and bone marrow (BM). The aim of study was evaluation of *NKX2*, *STEAP1* and *CCND1* genes expression for BM involvement detection in Ewing sarcoma (ES) and primitive neuroectodermal tumours patients (PNET).
Material and Methods: Gene expression was estimated by multiplex quantitative real-time RCR in 59 BM samples obtained from ES and PNET patients with detected fusion gene transcripts (*EWS1-FLI* or *EWS1-ERG*) and in 8 BM samples of patients without malignancies.
Results: *NKX2* expression was not detected in normal BM, although *STEAP1* and *CCND1* expression was revealed in all BM samples from patients without malignancies. 17 BM samples from ES/PNET patients were considered true positive in case of tumour cells presence in BM smears or detection of fusion gene transcript by nested PCR. Expression of *NKX2* was detected in 16 samples, *STEAP1* and *CCND1* − in 17 positive samples. In negative BM samples mRNA *NKX2* was detected in 2 cases, while *STEAP1* and *CCND1* expression was noted in all 42 negative samples. The best diagnostic test performance values assessed by ROC-analysis were obtained for *NKX-2*. Positive predictive value (0.889), negative predictive value (0.976), diagnostic sensitivity (0.941), specificity (0.952) and overall correct prediction (OCP, 0.949) for this marker were high. OCP values for *STEAP1* and *CCND1* were relatively low (0.695 and 0.763), diagnostic sensitivity (0.824 and 0.588) and specificity (0.643 and 0.833 respectively) were also low. The only positive BM sample with absence of *NKX2* expression was obtained at the time of ES diagnosis. In this sample there were no tumour cells in BM smear but fusion gene transcript *EWS1-FLI* was detected. Simultaneous analysis of BM samples obtained from two another sites revealed expression both of *NKX2* and *EWS1-FLI* but microscopically these samples were negative.
Conclusions: *NKX2* revealed the best diagnostic test performance values for BM involvement detection in patients with Ewing sarcoma family tumours both at the diagnosis and during treatment. *STEAP1* and *CCND1* showed remarkably low diagnostic characteristics and their application for marrow disease detection is inappropriate.

4123 POSTER

"The Breathing Tree Project" Biofeedback and Stress Mitigation in Children With Cancer

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Background: The high increase in the percentage of the survival rate of childhood cancer has led experts in the field to devote their attention not only to the healing of children with cancer, but also to guarantee the highest possible quality of life during and after this experience.
The international research literature seems to indicate that hospitalization can present anxiety and stress in children which as a consequence requires good coping skills.
The present study aims to evaluate the effectiveness of biofeedback training in reducing anxiety levels in a group of children suffering from cancer.
Biofeedback is considered to be an efficient method for stress mitigation in children.
Material and Method: *Hypothesis:* We hypothesized that the application of five weekly sessions of biofeedback training has a significant effect in reducing levels of anxiety experienced by a group of children suffering from cancer diseases. We hypothesized also that this effect is maintained with a follow-up treatment a month after the end of training.
Participants: Children and adolescents aged 6−18 who have experienced at least one hospitalization will be included in this study. Participants should be aware of their diagnosis for at least a month, and the whole process of research must be completed before the stop therapy.
Assessment tool: to assess the anxiety levels of participants will evaluated on the Test of Anxiety and Depression in children (TAD).
Training: The biofeedback training will be carried out using an instrument consisting of three sensors, which are applied to the fingers and connected to a PC that collects the following parameters: heart rate variability and skin conductance, and a software that provided a series of exercises that offer a very pleasing visual stimuli that may lead to a mood change.
Procedures: Study participants will be chosen among patients followed by the paediatric haematology-oncology ward of Brescia's Civil Hospital. Participants will be given a TAD test as an initial assessment, then training sessions start. At the end of the five appointments, the TAD will be re-administered as a final evaluation.
After one month of completion of the training, the TAD will be repeated as a follow up to assess the maintenance of the effectiveness of training.
Expected results: It is expected that training of biofeedback has a significant effect in reducing levels of anxiety experienced and that this effect is maintained until follow-up.

4124 POSTER

Robotic Stereotactic Radiotherapy in the Management of Pediatric Patients With Benign and Malign Lesions

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Background: We evaluated our therapeutic results with robotic stereotactic radiotherapy (SRT) in the management of pediatric patients.
Material and Methods: Between June 2007 and August 2010, 30 pediatric patients were treated with robotic SRT in our department. The median age was 9.5 years (range, 3−16 years). Twenty-five patients had lesion in cranial location, and five patients had extracranial disease. There were 19 patients with central nervous system tumours, 5 with arteriovenous malformation (AVM), 1 with histiocytosis, 2 with sarcoma, 1 with neuroblastoma, 1 with malignant rhabdoid tumour, and 1 with germ cell tumour. The median target volume was 16.6 cm^3 (range, 0.3–1233 cm^3). The median marginal total dose was 25 Gy (range, 8−30 Gy), and the median marginal isodose line was 79% (65−90%). Robotic SRT delivered with CyberKnife® (Accuray Inc, Sunnyvale, CA). The median number of beams was 179, conformity index was 1.6, and homogeneity index was 1.3. Three patients were treated with a single fraction, and 27 patients were treated with a fractionated stereotactic radiosurgery. Nine patients (30%) did require general anesthesia during robotic SRT.
Results: The median follow-up time was 8 months (range, 1−41 months). Complete response was observed in 5 patients, partial response was seen in 5 patients, stable disease was observed in 13 patients and remaining 7 patients had progressive disease. Eleven patients died due to disease. One patient with anaplastic astrocytoma developed brain necrosis as a late complication in the fifth months of follow up. None of the patients died due to treatment related complications.
Conclusions: Our initial results with robotic SRT in pediatric age group are promising. Our SRT scheme was generally well tolerated, and general anesthesia was not required in most of the patients. However, long term follow-up of these children is required to see late effects of SRT in pediatric population.

4125 POSTER

Nimotuzumab and Vinorelbine Concomitantly to Radiation and as Maintenance for Diffuse Pontine Glioma in Childhood − Promising Results on a Series of 13 Patients

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Background: Prognosis for diffuse pontine glioma of childhood is owful with median PFS and OS around 6 and 9 months, respectively. After joining a previous trial with nimotuzumab and radiation and continuing for a total of 37 patients from January 2006 to June 2009 according to this new combination, we obtained a median PFS of 7 months and a median OS of 11 months, i.e. thus entirely consistent with best literature data and those reported previously by ourselves (Massimino 2008). All treatment

was given on an outpatient basis and without any side effects correlated with the use of nimotuzumab. Two/37 children were alive without tumour progression 21 and 29 months after their diagnosis.
Materials and Methods: In a subsequent mono-institutional experience, with a view to exploring add-on strategies, we began a pilot protocol on a compassionate case-by-case basis using nimotuzumab together with vinorelbine, combined with radiation, and adopting consolidation courses with the same timing as in the previous international radiation plus nimotuzumab protocol. Vinorelbine was adopted at a dose of 20 mg/sqm/weekly together with nimotuzumab at the standard dose of 150 mg/sqm during the 6 weeks when radiotherapy was delivered, and 25 mg/sqm in any other week, with the same dose of nimotuzumab during the consolidation courses, planned until tumour progression or for a total of two years.
Results: We have so far treated 13 children, 7 males and 6 females, with an age range of 2−13 years, enrolled according to the standard MRI inclusion criteria. After a median follow-up of 10 months (range 3- 20), 11/13 were alive, their PFS at 9 months was 47±15% and their OS at 12 months was 92±7%. Median PFS was 9 months and median OS has not been reached. According to MRI evaluation, in 12/13 children evaluable for response, 9 had partial remission and 3 stable disease, 100% had symptom amelioration.
Conclusions: The nimotuzumab/vinorelbine combination was very well tolerated, with no acute side-effects. As in the case of nimotuzumab alone, all children were treated on an outpatient basis. The observation time for this new series is long enough to give the impression that this combination has promise, with statistically significant differences with previous reported experiences as far as OS.

4126 POSTER

Hsp70 Stress Protein is a Promising Tool in the Treatment of Brain Tumours in Children

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Background: Primary malignant brain tumours are the second common malignancies, after leukemia, and represent 20% of all children's cancers. In the majority of cases the therapy includes surgery followed by radiotherapy and concomitant chemotherapy. Despite this aggressive multimodal approach, the prognosis for patients is poor. As such, investigative therapies could be based on immunomodulatory activity of molecular chaperons, particularly Hsp70.
Material and Methods: To define *in vivo* response we used a model of intracranial C6 glioma in rats, which were intratumorally injected with Hsp70. To characterize immune effect we used cytotoxicity assay of spleen lymphocytes (CTL-test), immunohistochemistry of brain sections for CD3+, CD4+, CD8+ cell infiltration, tumour volume assessment by magnetic resonance imaging, MRI. All animals were followed for survival.
Patients (n = 10) with diagnosis of malignant brain tumour following operation were treated by intratumoral administration of five doses of Hsp70 (at 500 μg for one injection) − totally 2.5 mg of protein. Immunological assays including lymphocyte subpopulations measurement, cytokine levels (INFgamma, TNFa, IL-4, IL-6, IL-10), cytotoxic activity of NK-cells before and after treatment were made. The courses were performed on the base of informal agreement and the resolution of the Ethical Committee of the Russian Neurosurgical Institute by A.L.Polenov MHSD RF.
Results: *In vivo* intracranial delivery of Hsp70 increased survival rate of rats from 18.5±2 till 35±3 Days ($P > 0.001$) depending on a mode of the chaperone injection. The delay was accompanied by C6-specific CTL response, infiltration with CD3+, CD4+ and CD8+ cells both in the area of injection and in tumour Itself. The hindrance of tumour volume growth according to MRI in Hsp70 treated group was also observed.
Intracranial delivery of Hsp70 in patients was not associated with any evidence of toxicity or serious adverse effects. One patient had an objective clinical response as revealed by MRI. After treatment we observed the elevated levels of INFgamma, TNFa, T-cell lymphocytes (CD3+CD4+, CD3+CD8+, CD3+HLA DR+). Cytotoxic activity of NK-cells was not significantly changed.
Conclusions: Our data provide the evidence of the feasibility, safety, and *in vivo* immunomodulatory activity of Hsp70 in patients. The results suggest that the target delivery of the chaperone Hsp70 can become a useful therapeutic strategy against malignant brain tumours.

4127 POSTER

Myocardial Performance Index − an Early Indicator of Subclinical Functional Anthracycline-induced Alteration in Children With Acute Lymphoblastic Leukemia

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Background: The risk of anthracycline-induced cardiovascular disease remains a major concern in children with acute lymphoblastic leukemia (ALL), giving the growing number of survivors. We investigated on the role of serum concentrations of biomarkers (cTnT and NT-pro-BNP) and myocardial performance index (MPI), as predictors of subclinical functional anthracycline-induced alterations.
Methods: All children admitted to our Pediatric Oncology Department from January 2007 to October 2008 with the diagnosis of ALL receiving anthracyclines as part of their therapy were enrolled in this study. Following informed consent, cTnT and NT-pro-BNP were evaluated in all patients at diagnosis, before the anthracycline therapy, 2 and 24 hours following every anthracycline administration. Physical examination, ECG and detailed echocardiography were performed at diagnosis, 24 hours after every anthracycline course and 12 months after the end of the chemotherapy.
Results: 19 children with standard-risk ALL were evaluated. The mean age was 6 years (range 10 months-14 years). The cumulative doxorubicin dosage was 240 mg/m^2, according to the AIEOP ALL 2000 protocol. None of 19 patients developed clinical signs or symptoms of congestive heart failure. With increasing cumulative dosages of anthracyclines a significant increase was seen in MPI (p 0.014). This increase was statistically significant both at a cumulative dosage of 240 mg/m^2 (p 0.018) and at the follow-up (p 0.05), compared to baseline, while the median NT-pro-BNP did not changed significantly during the treatment. In all patients the cTnT levels remained negative in all samples.
Conclusion: A proportional increase of MPI was observed with increasing anthracycline doses, while cTnT and NT-pro-BNP levels did not change significantly during the therapy.
MPI is a sensitive and reliable parameter, able to detect subclinical cardiac dysfunction in children receiving anthracycline-based treatment. More prolonged follow-up is required to establish the impact of MPI on the prediction of possible cardiac dysfunction.

4128 POSTER

Procedural Analgo-sedation Role in Reducing the Incidence of Traumatic Lumbar Puncture in Children With Acute Lymphoblastic Leukemia

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Background: Invasive procedures, such as the lumbar puncture (LP), can cause anxiety and pain in children undergoing treatment for acute lymphoblastic leukaemia (ALL), often leading to traumatic lumbar punctures (TLPs). TLP increases the risk of central nervous system (CNS) relapse. The objective of the study is to evaluate the safety and efficacy of procedural analgo-sedation and its role in reducing risk of TLP in children with ALL.
Patients and Methods: From September 2007 to November 2008 we performed a total of 225 LPs in 25 children with ALL, treated according to AIEOP ALL 2000 protocol. Thirteen males and 12 females were included, with a median age at diagnosis of 5.7 years (age range, 2 months to 14 years). All the procedures were performed under deep sedation, using Propofol and Ketamine. Vital parameters were monitored thought procedures and possible side effects were recorded. The efficacy of deep sedation was evaluated using Ramsay and CHEOPS scales. Cerebrospinal fluid (CSF) was collected for chemical and cytologic examinations. LP was defined as traumatic if 10 or more erythrocytes per cubic millimeter were found in CSF.
Results: In all patients a satisfactory sedation and analgesia were achieved. The mean awakening time was 25±10 minutes. The evaluation of vital parameters didn't show any significant variation compared to baseline values. No apnoea episode was recorded and O2 Saturation ranged between 94% and 99%. No side effects related to the drugs utilized were recorded. The mean Ramsay and CHEOPS scores were 6.15±1 and 5.5±0.5 respectively. Out of 225 LPs performed under analgo-sedation only 3 (1.3%) resulted traumatic.
Conclusion: Procedural analgo-sedation was safe and efficacious, improving comfort and quality of life of children with ALL. Moreover, deep sedation reduce the risk of TLP that, especially at diagnosis, increases the risk of CNS relapse, negatively influencing the patient outcome.