4110 POSTER

Prognostic Significance of Mediastinal Involvement in Childhood Lymphoblastic Lymphoma

S.V. Semochkin[1], S.S. Kulikova[1], D.V. Litvinov[2], E.V. Inyushkina[2], N.V. Myakova[2], K.L. Kondratchkik[2], L.V. Baydun[3], D.M. Konovalov[4], D.A. Peregudov[2], E.V. Samochatova[2]. *[1]Federal Scientific Clinical Center of Pediatric Hematology Oncology and Immunol, Adolescent and Young Adult Hematology, Moscow, Russian Federation; [2]Federal Scientific Clinical Center of Pediatric Hematology Oncology and Immunol, Pediatric Hematology, Moscow, Russian Federation; [3]Federal Scientific Clinical Center of Pediatric Hematology Oncology and Immunol, Laboratory Hematology, Moscow, Russian Federation; [4]Federal Scientific Clinical Center of Pediatric Hematology Oncology and Immunol, Pathology, Moscow, Russian Federation*

Lymphoblastic lymphoma (LBL) is an uncommon malignancy accounting for approximately 2% of all non-Hodgkin's lymphomas (NHL). Most of the studies carried out on a small number of patients (pts) and the significance of prognostic factors is poorly understood. The purpose of this study was to investigate the effect of individual features on the outcome.
Methods: 58 patients (m − 40, f − 18) were enrolled from May 1991 to August 2008. Fifty-two (90%) patients were treated with NHL-BFM 90 or 95 protocols for non-B-NHL (leukemia-type) and 6 (10%) − NHL-BFM 90 (NHL-type).
Results: Median age at time of presentation was 11.0 (range 1.5–21.6) years. 45 (90%) patients have a T-cell immunephenotype. 53 (91%) had advanced (III, IV) stage. The presenting sites of T-LBL included mediastinal mass − 35 (78%) and lesion of bone marrow − 13 (29%). The complete response (CR) rate was 94 and 83% for non-B-NHL and B-NHL treatment respectively. 5-years event free survival (5 y-EFS) was 0.80±0.06 (median of observation 4.1 years) and 0.67±0.19 (5.1 years) respectively ($p > 0.05$). 5-years overall survival (5 y-OS) was 0.85±0.05 and 0.80±0.06 respectively ($p > 0.05$). The situation without mediastinal involvement was a factor unfavorable prognosis for T-LBL: 5 y-EFS − 0.56±0.17 vs. 0.90±0.05 ($p = 0.036$). Sex, age, increased LDH, slow or fast therapy response, involvement of the central nervous system or bone marrow did not affect the prognosis ($p > 0.05$).
Conclusions: The absence of mediastinal involvement is a factor unfavorable prognosis for childhood T-LBL treated with BFM protocols.

4111 POSTER

Clinical Importance of Methotrexate Pharmacokinetics and Pharmacogenetics in Pediatric Osteosarcoma

M.Z. Hegyi[1], A. Gulacsi[1], A.F. Semsei[2], E. Cságoly[2], D. Erdelyi[2], C.S. Szalai[2], G.T. Kovacs[1]. *[1]Semmelweis University, II. Department of Peadiatrics, Budapest, Hungary; [2]Semmelweis University, Department of Genetics Cell- and Immunobiology, Budapest, Hungary*

Introduction: High-dose methotrexate (HD-MTX) with leucovorin rescue is widely used to treat osteosarcoma, which predominantly affects children. The human gamma-glutamyl hydrolase (GGH) is important in the antifolate-resistance in the tumour cells. The reduced folate carrier(RFC1)gene encodes the major methotrexate transporter. Our objectives were to analyze the relationship between exposure to HD-MTX and toxicity, and to assess correlations between pharmacokinetic parameters, toxicity and polymorphisms of the GGH promoter (**GGH −401C>T**) and the the reduced folate carrier **(RFC1 G(80)A)** in children with osteosarcoma.
Methods: Pharmacokinetic data of 112 patients treated with 1029 HD-MTX courses were evaluated. Pharmacokinetic parameters (MTX clearance and AUC) were calculated based on methotrexate serum levels measured at 6, 24, 36, 48 hours after the initiation of the infusion by high pressure liquid chromatography (HPLC) technique. Clinical data (the highest serum GPT, GGT, bilirubin values and the lowest number of granulocyte, thrombocyta and serum protein levels in the first two weeks after methotrexate treatments) were collected by retrospective chart review. Toxicity parameters were categorized according to Common Toxicity Criteria. Data were analysed by Student's t-test, Mann-Whitney U test and chi square test (StatSoft's STATISTICA v8.0). The polymorphisms were determined by a PCR-RFLP method using DNA extracted from peripheral blood of 72 children among the 122 patients.
Results: Patients with serious hepatotoxicity had significantly higher peak MTX concentrations ($p = 0.002$), 48 h MTX serum levels ($p = 0.0034$) and AUC_{0-48} ($p = 0.00001$), and significantly lower MTX clearance ($p = 0.0001$). Patients with serious bone marrow toxicity had significantly higher 24 h MTX serum levels ($p = 0.000012$). Nephrotoxicity was associated with higher 24 and 48 h MTX serum levels and higher AUC_{0-48} ($p < 0.00001$). The incidence of serious acute hepatotoxicity was less frequent ($p = 0.0033$) and drug serum levels were significantly lower in the cellular elimination phase ($p = 0.0003$ at 48 hours) in patients homozygous for the GGH −401T allele than in the group with −401CC or CT genotypes. The frequency of serious acute hepatotoxicity was significantly higher ($p = 0.001$) in patients with RFC1 80AA genotype than in those who carried the G allele. This difference was even higher between patients with RFC1 80AA plus GGH-401CC+CT genotypes and patients with other genotypes ($p = 0.00005$).
Conclusion: Higher MTX exposure leads to more frequent occurence of toxicity. Patients homozygous for the GGH −401T allele had less hepatotoxicity and faster methotrexate elimination compared to those with −401CC or CT genotype. The hepatotoxicity was more frequent in patients homozygous for the RFC1 80A allele than in those who carried the G allele and the difference was intensified without the protective effect of GGH −401TT genotype.

4112 POSTER

Treatment Outcome of Children With Hepatoblastoma

K. Josip[1], M. Anicic[1]. *[1]Clinical Hospital Centre Zagreb, Pediatric Hematology and Oncology, Zagreb, Croatia*

Background: Hepatoblastoma is the most common malignant primary tumour of the liver in children. The prognosis of hepatoblastoma in children has significantly improved over the last 20 years. This is attributable to improved multidisciplinary input including specialist pediatric hematology-oncology and surgery.
Objective: To review our institutional experience with hepatoblastoma.
Patients and Methods: Files of children treated at our hospital between 1995–2010 with the diagnosis of hepatoblastoma were reviewed for clinical characteristics and treatment results. All patients presented a palpable abdominal mass. Ultrasound, CT and/or MRI were used to assess site and resectability of tumours. All patients underwent diagnostic biopsy. 9 children (5 male and 4 female, median age at diagnosis was 2.3/ 1−3.5 y) with hepatoblastoma (3 had lung metastases) have been treated according to SIOPEL protocol with pre-operative chemotherapy, surgery and post-operative chemotherapy. One was treated only with chemotherapy (surgery wasn't possible).
Results: The remission has been achieved in all patients; 2 patients died in relapse. 7 patients are still alive in the first remission (also 3 with lung metastases and 1 treated only with chemotherapy). Serious side effects were not noticed (only 1 cardiomyopathy). Secondary malignancies did not occur in any of patients.
Conclusion: Combined modality therapy is optimal treatment for the majority of children with hepatoblastoma. New treatment strategies using innovative approaches are still needed to further improve treatment results.

4113 POSTER

Pharmacokinetics and Toxicity of 5 G/m^2 MTX Treatments in Children With ALL

K. Csordas[1], M. Hegyi[1], O. Eipel[1], M. Csoka[1], J. Muller[1], G.T. Kovacs[1]. *[1]Semmelweis University, 2nd Department of Pediatrics, Budapest, Hungary*

High-dose methotrexate (MTX) is the part of treatment for different childhood malignancies. It has been used for many years, however the exact dose is not clearly defined.
The aim of our study was to analyse the pharmacokinetic data and toxicity of 5 g/m^2 MTX treatments in children with acute lymphoblastic leukemia (ALL) treated according to BFM 1995 and 2002 protocols at the 2nd Department of Pediatrics at Semmelweis University between 1998–2006.
Patients and Methods: 43 patients were treated with 5 g/m^2/24h intravenous MTX. Mean age of the patients was 7.02 years (0.45–17.95). 147 MTX infusions were analysed. Serum MTX and 7-OH-MTX levels were measured with HPLC at 24, 36, 48 hours. Delayed elimination was registered. We calculated AUC and half-life time at the first and at the terminal phase of elimination. Considering the toxicity we measured the serum ALAT, ASAT, bilirubin, creatinine, protein levels before therapy and one day, two days and one week after treatment. Correlations between pharmacokinetics and toxicity were calculated using Mann-Whitney-test and Chi squre-test after testing normality.
Results: MTX elimination showed two-compartment model, with a fast phase between 24. and 36. hours, and a slow phase between 36. and 48. hours. Mean half-life time in the first phase (T1) was at 30 hours (SD: ±0.4), in the terminal phase (T2) it showed greater difference: the mean time was at 45 hour (SD: ±3.1). There was no significant correlation between AUC, T1, T2, 24.h, 36.h MTX and 24.h, 36.h, 48.h 7-OH-MTX and the toxic parameters. We found significant correlation between 48.h MTX and serum ALAT ($p = 0.029$), serum bilirubin ($p = 0.006$) levels elevation. Delayed elimination correlated with serum bilirubin level elevation ($p = 0.032$).
Conclusion: Serum MTX levels show great inter- and inpatient differences. Higher 48. hour plasma concentration and delayed elimination show strong

correlation with the appearance of acute hepatoxicity. It is important to monitore drug pharmacokinetics to be able to use intensive supportive care if it is necessary to avoid serious adverse effects.

4114 POSTER

Monitoring of Bone Marrow Clearing by an Automated Imaging Device (Immunofluorescence Plus Genetics) Identifies Different Risk Groups in Neuroblastoma Patients Over 18 Months

I.M. Ambros[1], U. Pötschger[2], A. Ziegler[1], D. Modritz[2], H. Gadner[3], R. Ladenstein[4], P.F. Ambros[5]. *[1]C.C.R.I., CCRI Tumour Biology, Vienna, Austria; [2]C.C.R.I., CCRI SiRP, Vienna, Austria; [3]St. Anna Kinderspital, CCRI, Vienna, Austria; [4]St. Anna Kinderspital and CCRI, CCRI SiRP, Vienna, Austria; [5]CCRI St. Anna Kinderkrebsforschung, CCRI Tumour Biology, Vienna, Austria*

Background: Reliable response criteria are still lacking in stage 4 neuroblastomas. We hypothesized that the dynamics of BM clearing mirrors the response to cytotoxic treatment and is thus able to identify prognostically differing subgroups of stage 4 patients.
Patients and Methods: BM samples from 81 stage 4 patients registered in two neuroblastoma Trials were tested with a fully automatic fluorescence based device combining GD2 based immunocytology and molecular-cytogenetic analyses of identical cells (automatic immunofluorescence plus FISH, AIPF). 44 patients (age 0 to 239 months, 219 BM specimens, median observation time 8.2 years) met the inclusion criteria (BM specimens at diagnosis and given time points during treatment and genomic information on the primary tumour) with a complete data set.
Results: BM clearing after 2 to 4 chemotherapy cycles was achieved by 28 patients (63.6%) and was significantly associated with overall survival (OS) in patients above 18 months at diagnosis ($p < 0.0002$, Logrank test) but not in the younger age group. Stage 4 patients below 18 months had a good prognosis irrespective of BM clearing and tumour genetics. In younger patients, none of the genetic markers showed a correlation with OS. MNA was associated and intact 11q showed a trend towards association with BM clearing ($p < 0.3$ and $p = 0.0735$, both Fisher's Exact Test).
Conclusion: The determination of BM clearance reaches the so far highest prognostic impact in stage 4 neuroblastoma patients over 18 months of age making accurate BM monitoring an important tool for risk assessment in this patient group.

4115 POSTER

Thiopurine-S-Methyl-Transferase Gene Polymorphisms and Antimetabolite Drug Toxicity in Children Treated for Acute Leukemia and Non-Hodgkin's Lymphoma

M. Kantar[1], B. Kosova[2], C. Aktan[2], E. Inalpolat Yucel[2], G. Koturoglu[3], Z. Kurugol[3], N. Cetingul[1]. *[1]Ege University School of Medicine, Pediatric Oncology, Izmir, Turkey; [2]Ege University School of Medicine, Medical Biology, Izmir, Turkey; [3]Ege University School of Medicine, Pediatrics, Izmir, Turkey*

Background: Thiopurine-S-Methyl-Transferase (TPSMT) enzyme activity may be changed due to different mutations in its allelles. Heterozygous or homozygos TPSMT mutations may result in more drug toxicity. Major adverse effects of 6-mercaptopurine and 6-thioguanine are hematologic and hepatic toxicities in the treatment of acute leukemia and non-Hodgkin's lymphoma (NHL).
We aimed to study TPSMT polymorphisms in a pediatric population who are treated for acute leukemia and NHL, and to relate this polymorphisms with hematologic and hepatic toxicity observed during maintenance treatment.
Materials and Methods: The study included 49 patients with median age 8 years (2−17) (30 male, 19 female). 40 patients had ALL, 3 AML, and 6 lymphoblastic lymphoma. The control group consisted of 84 children with median age 9.5 years (34 male and 50 female).
Eight polymorphisms of TPSMT gene were examined by RT-PCR method in the extracted DNA from peripheral blood of the study and the control groups. Grade III or IV hematologic (Hb, WBC, platelet counts) and hepatic toxicity (serum ALT, AST, bilirubin levels) data were recorded using patients charts. Chi-square test was used in statistical analysis.
Results: We have found that the patients to have generally wild-type TPMT (*1) in 87.3%, TPMT*2 (G238C) in 2%, and TPMT*3B polymorphisms (G460A) in 20.4%. The other polymorphisms including TPMT*3A, TPMT*3C (A719G), TPMT*3D, TPMT*4 (G−A), TPMT*5 (T146C), TPMT*6 (A539T) and TPMT*7 (T681G). In the control group, wild-type TPMT (*1) was in 98.8%, TPMT*3B in 1.2%. Other polymorphisms were not detected. In comparison, the patients were found to have less wild- type TPSMT, but more TPMT*3B polymorphism ($p = 0.0001$ and 0.0001). We did not find any relationship between hematologic and hepatic toxicity and TPSMT gene polymorphisms.
Conclusions: We conclude that severe hematologic or hepatic toxicity in the maintenance treatment of acute leukemia and NHL is not related with TPSMT gene polymorphisms.

4116 POSTER

Glioblastoma Multoforme as a Second Malignant Neoplasm After Radio-chemotherapy for Pediatric Malignancies

L.I. Shats[1], M.B. Belogurova[1], O.G. Zheludkova[2], I.D. Borodina[2]. *[1]Clinical Hospital 31, Paediatric Oncology and Haematoogy, St. Petersburg, Russian Federation; [2]Institute of Paediatric Haematology Oncology and Immunilogy, Department of Neurooncology, Moscow, Russian Federation*

Background and Purpose: Reports of secondary high-grade glioma (HGG) in survivors of childhood cancer are limited.
Patients and Methods: We performed retrospective study in the group of patients with paediatric glioblastoma multiforme (42 patients) 6 children developed glioblastoma as a second malignancy (age 7−15 years, median 12 yrs). We report on 6 patients (2 males, 4 females) treated in childhood for a primary cancer. All patients previously had received radiation and chemotherapy for either acute lymphoblastic leukaemia (n = 5) or medulloblastoma (n = 1). Children developed glioblastoma 5−10 years thereafter (median 6 yrs). Median of dose of primary cranial irradiation was 18 Gy. Tumours were localized supratentorialy in 5 cases and was infratentorial (cerebellum) in one patient. Patients underwent surgery resection (2 − total, 3 − subtotal, 1- partial) followed by standard fractionated local radiation and chemotherapy. The dose of irradiation was 55−60 Gy (median 55 Gy). One patient progressed after subtotal resection and irradiation (2 mo after surgery). Five children received temozolomide (TMZ) as single-agent 150–200 mg/m^2 administered on 5 consecutive days every 28 days (number of courses 2−8, median 3).
Results: In 3 cases chemotherapy was stopped because severe myelotoxicity after 2−4 courses. Four patients have died and the median overall survival time was 16 months. Overall survival rate was 62.5±21.3% and 20.8±18.4% at 1 and 2 years after diagnosis respectively with follow up 4–24 mo with median 11 mo. Two patients are alive, but the only one without signs of disease progression.
Conclusions: Prognosis in secondary malignant gliomas in children is poor despite intense therapy. The risk of a severe myelotoxicity is high. ALL and medulloblastoma survivors must undergo systematic, long-term surveillance for early detection of intracranial neoplasms.

4117 POSTER

Intensive Chemotherapy and Temozolomide in Children With Newly Diagnosed Anaplastic Astrocytoma

L.I. Shats[1], M.B. Belogurova[1], G.G. Radulesku[1], O.G. Zheludkova[2]. *[1]Clinical Hospital 31, Paediatric Oncology and Haematoogy, St. Petersburg, Russian Federation; [2]Institute of Paediatric Haematology Oncology and Immunology, Department of Neurooncology, Moscow, Russian Federation*

Background: To evaluate the efficacy of temozolomide and poly-chemotherapy in a retrospective nonrandomized study among newly diagnosed children with anaplastic astrocytoma.
Patients and Methods: We analyzed 39 patients (age 4 mo to 17 years, median 9 years) from pediatric oncology departments in three institutions between January 1996 and April 2010 (at median follow-up of 13 mo, range 4–108 mo) in order to identify influence of different chemotherapy modalities in newly diagnosed paediatric anaplastic astrocytoma. All patients (pts) underwent resection (34pts) or biopsy (5 pts), followed by standard fractionated local radiation and chemotherapy. Two children were not irradiated because of the young age. Patients were divided into 3 groups according to treatment modalities. Eighteen pts received temozolomide (TMZ) as single-agent 150–200 mg/m^2 administered on 5 consecutive days every 28 days (number of courses 2−12, median 6), 12 children of the group also received concurrent chemotherapy during radiation with TMZ (75 mg/m^2/day). Second group of patients (18 pts) received polychemotherapy − one of two chemotherapy regimens: cyclophosphamide, etoposide, cisplatin and vincristine or ifosfamide, etoposide, carboplatin and vincristine (number of courses 2−26, median 8). Third group (3 pts) received polychemotherapy and TMZ sequentially because of the residual tumour after 4−8 courses of polychemotherapy (total number of courses 11−16).
Results: Overall survival rates in group of temozolomide were 51.6±12.5% and 43.0±13.0 (with a median of 20.0 mo) at 1 and 5 years after diagnosis, respectively. Overall survival rates in the group of polychemotherapy at 1 and 5 years were 77.8±9.8% and 61.1±11.5% (median undefined). Survival rates in both groups since 2 years after diagnosis were constant stable; nobody died or relapsed after two years of follow up. The log-rank test in OS between the two groups was not statistically significant ($P = 0.14$).