402 POSTER

Incidence of TEL/AML1 fusion genes in children with ALL: Risk assessment by BFM and MRD criteria

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Background: The fusion of TEL and AML1 genes is the most common chromosomal translocation in childhood acute lymphoblastic leukemia (ALL). The incidence of this translocation is about 25% at diagnosis and was found to be associated with an excellent prognosis. There are controverse data about the incidence at relapse in different treatment protocols. Sofar, no data have been reported on the response to chemotherapy by molecular methods (minimal resiudal disease, MRD) as one of the most powerful prognostic factors.

Aim of the Study: To analyse children with ALL at diagnosis and relapse for the incidence of TEL/AML1 and compare MRD-based risk stratification with clinical outcome.

Patients and Methods: 53 children with ALL treated according to ALL-BFM 90/95 protocols were included. TEL/AML1 translocation was analysed by FISH. Leukemia-specific antigen receptor gene rearrangements were used for MRD detection. MRD risk groups are: low risk (LR) no detectable MRD after induction, intermediate risk (IR) MRD 10-3 before consolidation, high risk (HR) "10-4 before consolidation therapy.

Results: After a median observation time of 5.7 years 13 patients suffered a relapse. At diagnosis 14 leukemias were TEL/AML1 positive. Of these 3 children suffered a systemic relapse. Children with TEL/AML1 negative ALL who relapsed remained TEL/AML1 negative.

According to BFM risk stratification 4 and 10 children with TEL/AML1 positive leukemias were treated according to BFM SRG and MRG protocols, respectively. The three relapses occurred in the MRG patients. When MRD risk stratification was applied 5, 7 and 2 children were in the MRD based SR, IR and HR group. Relapses occurred in the IR (2 patients) and HR group (1 patient).

Conclusion: The incidenc of TEL/AML1 positive leukemias is identical at diagnosis and at relapse in children treated according to ALL-BFM90/95 protocols. Children with relapse of the TEL/AML1 positive leukemia were not in the MRD-based LR group, in which no relapses occurred.

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Ex vivo purging using MC540 photoirradiation therapy enhanced by amifostine (WR 2721)

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Photodynamic treatment using MC540 as a photosensitising dye has a potential use in the purging of neoplastic cells from autologous bone marrow grafts. Aminothiols exert cytoprotection on normal tissues from systemic chemotherapy. This study was designed in order to investigate the effect of Amifostine (WR-2721) on leukemic and normal bone marrow cells after MC540 photoirradiation therapy. Bone marrow cells from children with acute leukemias (AL) at initial diagnosis and in remission under maintenance chemotherapy as well as HL-60 leukemic cell line were incubated with Amifostine (1.5 mg/ml) for 15 min and then with MC540 (20 g/ml) for 1 hour. Afterwards, they were exposed to different Argon Laser 514 nm doses. Cell suspensions which were not incubated with Amifostine were used as controls. Cell survival was estimated with trypan blue supravital stain following a 24 hour incubation and leukemic cell line has been studied in continuous cell cultures of 4 weeks duration. The survival of normal bone marrow progenitors has been estimated by colony formation assay in semisolid cultures. Our results showed that Amifostine: 1) has enhanced the photokilling effect of MC540 on both HL-60 cell line and fresh bone marrow leukemic cells 2) significantly protected bone marrow precursors from children with AL under chemotherapy from cytotoxicity induced by photodynamic treatment (39.05 + 4.11% vs 62.9 + 9.9%, p = 0.008), 3) has improved the survival of bone marrow committed progenitors (24.17 + 8.8% vs 5.67 + 1.7%, p = 0.08 for CFU-E, 76.33 + 39.14% vs 48.25 + 21.49%, p = 0.3 for CFU-GEMM and 44.69 + 11.2% vs 29.15 + 9.6%, p = 0.15 for CFU-GM). These differences were found to be statistically significant only for BFU-E (60.27 + 15.37% vs 18.82 + 4.64%, p = 0.017) colony formation. In conclusion, Amifostine (WR-2721) seems to enhance the photokilling effect of MC540 photoirradiation on leukemic cells and in addition to the above action exerts cytoprotection upon normal bone marrow cells; thus this agent could play a significant role in clinical use of MC540 mediated phototherapy.

404 POSTER

Radiotherapy for unresectable or marginally resectable osteosarcoma

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To assess local control following radiotherapy for unresectable osteosarcoma.

Between April 1967 and October 1997, 16 patients with osteosarcoma received radiation therapy to 19 sites. RT alone was used in 12 sites, and combined with surgery in 7. The median dose was 60 Gy (range, 27.5 to 82.8) using conventional fractionation (1.8 to 3 Gy once daily, QD) in 6 cases and hyperfractionated RT (1.1 to 1.5 Gy, twice daily, BID) in 13 cases. Response to chemotherapy (CT) among 14 treated prior to RT was progressive (PD, n = 7) or stable (SD, n = 7). Concurrent chemotherapy was delivered during 10 courses. At time of analysis, 9 of 16 patients are alive at a mediam time of 78 months.

Overall, 8 of 19 sites remained free of progression through last follow-up or death; median interval of control is 5.1 months (range, 0.2 to 355). Local tumor control has been achieved in 2 of 12 cases with RT alone (60 Gy delivered in 7), 3 of 3 with pre-operative RT and 3 of 4 with post-operative RT. Local tumor control has been maintained in 5 of 7 cases of SD with prior chemotherapy and 2 of 7 cases of PD. Six of 10 patients with concurrent CT, and 2 of 9 without CT have evidenced local control. Neither fractionation nor treatment interruptions affected outcome.

Local control of osteosarcoma with conventional RT, in absence of radical resection is a significant problem. In marginally resectable patients, adjuvant radiotherapy may improve local control. While hyperfractionated RT to 60 to 70 Gy for unresectable disease may improve normal tissue tolerance, tumor control remains poor.

405 POSTER

Ewing's sarcoma: A review of treatment outcomes and morbidity in patients aged 16 and less treated in British Columbia between 1980 and 1992

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Purpose: To review the outcome of treatment of Ewing's sarcoma in British Columbia for patients (pts) aged * 16 yrs after a minimum of 7 yrs follow up.

Methods: A retrospective chart review of these pts (recorded on a provincial registry) seen and treated between 1980 and 1992.

Results: There were 33 pts. Age at diagnosis ranged from 2 to 16 yrs. Median age at diagnosis was 11.5 yrs. All pts received adriamycin containing combination chemotherapy. The maximum dose of adriamycin given was 450 mg/m^2. 28 pts received XRT alone and 5 surgery alone as local therapy. The minimum follow up of these pts is 7 yrs. Average survival is 8.3 yrs. The mortality rate is 39% (13 pts). 27% (9 pts) died from recurrent disease. 1 died due to early complications related to chemotherapy, 2 died due to adriamycin induced cardiomyopathy, 1 due to chest wall deformity secondary to surgery leading to respiratory failure. Long term complications: 5 pts have left ventricular dysfunction requiring medication and 1 pt is surviving after heart transplant. 1 pt suffered spontaneous bone fracture after trivial injury within the previous XRT field. 4 pts with head and neck tumors have significant facial hypoplasia, dental decay and trismus. There have been no cases of second malignant neoplasm arising in any pts yet.

Conclusion: Long term complications in survivors of Ewing's are significant. Multidisciplinary follow up is mandatory.

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Astrocytomas of the cerebellum in children

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Purpose: To analysis the effect of postoperative radiotherapy in childhood cerebellar astrocytoma.

Methods: Between January 1978 and December 1997, 100 children (aged 17 years or younger) with astrocytoma were treated. We have retrospectively reviewed 39 patients with diagnosis of astrocytoma of the cerebellum. Of the 39 patients in this study, 22 were girls and 17 were boys. Their ages ranged from 2 to 17 years of age, with a mean of 10 years. Presenting signs and symptoms were papilledema (81%), headache

(80%), nausea/vomiting (78%) and ataxia (64%). Subtotal tumor excision was performed in 34 patients and biopsy was performed in only 5 patients. Histopathologically, each case was reviewed. The patients' tumors were classified as either microcystic or diffuse. Twenty-seven of the 39 patients (69.2%) had microcystic tumor; the remaining 12 (30.8%) had diffuse tumor. Of the 27 cystic tumors, 25 (92.6%) were subtotally excised. This compares to 9 (75%) of the 12 diffuse tumors amenable subtotally excised. According to Kernohan Grading, 32 patients were grade III and 7 patients were grade IIIIV. Low grade tumors were irradiated with a local field (1.82/4450 Gy) and children with high-grade tumors received a total brain irradiation (1.82/4045 Gy) followed by a boost irradiation 10 Gy, using a Cobalt-60 Unit. Follow up ranged from 6 to 121 months (median 49.9 months).

Results: Two, 5 and 10 year overall survival rates were 94%. Two, 5 and 10 year disease free survivals were 79.2% respectively. Of 34 patients whose tumors were subtotally removed, 7 (20.6%) recurred and 5 patients were performed biopsy and 3 (60%) recurred, with a mean follow-up of 3.8 years ($p = 0.023$). No correlation with survival could be determined for the gross appearance of rumor diffuse (90%) or cystic (100%) ($p = 0.15$).

Conclusion: Although there is no question that total surgical excision is the treatment of cerebellar astrocytomas, controversy arises as to the management of subtotally excised tumors. The issue of whether postoperative irradiation is beneficial.

407 POSTER

Mutations of RB-1 gene in children with leukemia and neuroblastoma

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Rb-1 is a tumor suppressor gene located in the 13q14 chromosome region and comprises of 27 exons. The RB-1 gene, code for a 110 KD product, which is a nuclear phosphoprotein acting as a cell cycle regulator and blocks the transition of normal cells from G0/G1 into the S phase of the cycle and it is normally expressed in hematopoietic cells. It is inactivated by deletions but more often by mutations. Point mutations may affect most of the exons, but have a certain predominance for exons 2024 and for their splicing sites. In hematopoetic malignancies, deletions or rearrangements of the RB-1 gene have been reported in 5 to 10% of acute leukemias, in adults. The aim of our study was to correlate the prevalence of RB-1 gene mutations with leukemia and neuroblastoma occurring in children. We studied archival bone marrow slides, dating from 1992 to 1996, from 26 children with leukemia (18 Acute Lyphocytic Leukemia, ALL and 8 Acute Myeloid Leukemia, AML) and 4 children with neuroblastoma. Exons 20, 21 and 22 were amplified using the PCR technique, resulting in products of 350 bp, 518 bp and 363 bp respectively. SSCP and heterodoublex analysis were performed to detect mutations. Due to its size, exon 21 was digested with NdeI restriction enzyme, resulting in 180 and 338 bp products. In exon 20, two samples of ALLs (11.11%), in exon 21, one of ALLs samples seemed mutated (5.56%) and in exon 22, four samples of ALLs (22.22%), had altered conformation. None of the AMLs or the neuroblastomas seemed to have mutations. Further analysis with sequencing is going to determine the actual percentage of mutations in all three exons. These data suggest that RB-1 gene could probably correlate with the etiology of acute lymphocytic leukemia and possibly used as a prognostic factor for the cause of the disease.

408 POSTER

Ochrobactrum anthropi bacteremia in children with central venous catheters

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Ochrobactrum anthropi is a gram-negative bacillus that has been isolated with increasing frequency last decade and associated with permanent central venous catheter-related bacteremias. Until 1993 only 15 cases of human infection due to O. Anthropi had been reported in the literature while during the period 1991–96, 9 cases of septicemia in 3 patients were identified in our department. The aim of this study was the estimation of frequency of O. Anthropi bacteremia in immunocompromised children with central venous catheters the last 2 years. During the period 1997July 1998 at our department, Ochrobactrum anthropi was isolated in 29 positive blood cultures (from Hickman and/or peripheral venous) of 9 children with malignant diseases (2 ALL, 3 solid tumors and 4 other hematological diseases). Seven of these children had central venous catheter (Hickman) and the positive blood cultures obtained from the catheter and peripheral venous as well. Although the efficacy of antibacterial chemotherapy in O. Anthropi infections is not defined in previous reports, in our cases the bacillus was resistant in vitro to b-lactam antibiotics and susceptible to imipenem, ciprofloxacine, amikacin and trimethoprim/sulfamethoxazole. According susceptibility tests the administration of imipenem or ciprofloxacine was efficacious treatment for 7 children while in two cases it failed to eradicate the organism and bacteremia relapsed after discontinuation of treatment which led to central venous catheter removal. These results indicate that the last years the incidence of O. Anthropi catheter-associated bacteremia increases and it is important to recognize it as causative agent and propose strategies for more effective control because it appears unpredictable multiple antibiotic resistance to many agents commonly employed in the empirical treatment of gram negative infections.

409 PUBLICATION

Changes of thyroid gland after combined treatment for Hodgkin's disease in children

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Purpose: To evaluate incidence and ways of treatment of thyroid gland's (TG) changes after therapy of Hodgkin's disease (HD) in children.

Methods: 36 patients were examined 113 years (31 of them 3 years) after chemotherapy and radiation therapy (total closes to the neck – 2546 Gy) for HD in childhood. The examination included measurement of levels of thyroid hormones, sonography of the TG and cytological or hystological examination of TG's nodular lesions.

Results: Impaired TG function was detected in 8 patients (22.2%): in 7 – hypothyreosis, in 1 – diffuse toxic goiter. In all patients with hypothyreosis and in 14 with normal TG function (total – 21 patients, 58.3%) hypoplasy of TG was detected with sonography. In 3 patients (8.3%) nodular lesions of TG were found (cytology: no signs of malignancy). In 1 patient papillary cancer of TG developed 11 years after neck irradiation, 45 Gy. L-thyroxin was used in cases of hypothyreosis with good effect. Thyroidectomy was performed for diffuse toxic goiter and TG cancer with subsequent therapy with L-thyroxin. In cases of benign nodular lesions follow up tactics was adopted.

Conclusion: The incidence of TG changes after treatment of HD in children is high. New approaches to treatment of HD are necessary so that to minimize those changes.

410 PUBLICATION

Metastatic brain involvement in children with Ewing's sarcoma

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Purpose: According to literature, brain metastasis (BM) is very rare in children with Ewing's sarcoma (ES). The aim of this study is to show the frequency of BM in patients with ES, as well as its dependency on the location of a primary lesion.

Methods: The review of 16 children (10 boys and 6 girls) with ES from 1993 until 1999 was completed. The ages of the children ranged from 3 to 17 years (median age: 11). 8 patients had 15 metastasis of different location, where brain metastatic involvement occurred in 33% of all metastasis. All BM were identified by imaging modality, 3 were histologicaly proven. At the time BM was diagnosed all patients had some CNS symptoms: headache (n = 3), headache and hemiparesis (n = 2).

Results:

Site of primary lesion	No. of patients	Metastatic involvement				
		brain	spine	lung	bone marrow	rib
Central: Pelvis	6	4	3	1	1	1
Rib	1	–	–	–	–	1
Peripheral: Femur	4	–	1	–	–	–
Humerus	1	–	–	–	–	–
Tibia	2	1	–	1	–	–
Fibula	2	–	–	1	–	–