

We aimed to determine the expression profiles of MRD markers in PB cell fractions and in PB stem cells (PBSCs) by RQ-PCR to determine cellular origins for illegitimate expressions.

Materials and methods: Granulocytes (gran.), T and B cells, monocytes (mono.), NK cells, and platelets (plts) from PB of healthy donors, and also samples of PBSCs were collected. RQ-PCR assays were based upon TaqMan technology using specific primers and probes. Total RNA was isolated and RNA samples were reverse transcribed by standard methods. Then, 50 PCR cycles were performed by ABI Prism 7700 sequence detector. All samples were studied in duplicates. Beta glucuronidase (GUS) was used as the 'housekeeping gene'. IMR-32 NBL cell line was examined as positive control. Samples with CT values >39.5 were accepted as negative. Results were compared to CT of GUS and ACT values (CT_{GUS} - CT_{Marker}) were calculated.

Results: PHOX2B was negative in all PB cell fractions. Number of positive samples for each marker in different cell fractions were: DDC 1/12 gran., 1/10 mono.; DBH 1/10 T cells; CHRNA3, 3/10 mono., 2/10 T cells; TH 5/12 gran., 8/10 mono., 3/6 NK cells; CHGB 11/12 gran., all T cells, 2/10 B cells, 4/10 mono., 3/10 plts; GAP43 1/12 gran., 1/10 T cells, all B cells, 1/10 mono., 2/10 plts. GD2, SNAP, STMN2 and STMN4 had varying degrees of positivity in all cell fractions. In PBSCs PHOX2B, DDC and CHRNA3 were negative, DBH was positive in 1/10, and others in ≥ 8/10.

Conclusions: PHOX2B was the best marker for MRD detection in NBL. In BM DDC, and in PB and PBSCs DDC, DBH and CHRNA3 also appear to be useful MRD markers. These results will guide future studies for more sensitive detection of MRD in NBL.

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POSTER

Could angiogenesis and the expression of Ki-67, p-53, p-27 and bcl-2 be prognostic factors in highly malignant brain tumors of childhood?

M. Moschovi¹, K. Karamolegou¹, K. Stefanaki², E. Koultsouki¹, G. Sfakianos³, P. Prodromou³, F. Tzortzatos-Stathopoulou¹. ¹Athens' University, Haematology/Oncology Unit, Athens, Greece; ²Children's Hospital 'Aghia Sophia', Pathology, Athens, Greece; ³Children's Hospital 'Aghia Sophia', Neurosurgery, Athens, Greece

Background: P-53, p-27 and bcl-2 are proteins encoded by genes that are implicated in cell cycle control, as well as in angiogenesis. Ki-67 is an index of cell proliferation. Aim of this study is the assessment of angiogenesis in highly malignant brain tumors in relation to the expression of Ki-67, bcl-2, p-53 and p-27. In addition, we investigated the possible association of these factors with the prognosis of the disease.

Material and methods: 29 children with embryonic type brain tumors enrolled in the study – 24 patients with Medulloblastoma (MB) and 5 with Atypical Teratoid/Rhabdoid Tumor (ATRT). The Streptavidin-Biotin immunohistochemical method was performed on tissue paraffin sections. The CD31 (YLEM[®]) monoclonal antibody was used in order to detect new blood vessels and determine their density. Monoclonal antibodies were also used for the detection of Ki-67 (DACO[®]), bcl-2 (Novocastra[®]), p-53 (DACO[®]) and p-27 (Novocastra[®]). All patients were followed up for 2 to 8 years (mean follow-up time: 4.7 years).

Results: Newly formed blood vessels were detected in all cases, whereas high density of new vessels was established in 12 MB and 3 ATRT. High levels of Ki-67 index (>25%) were detected in 87% of MBs. MBs with high density of new vessels presented with concomitant increased expression of Ki-67. The majority of MBs (79%) had high expression of p-27. P-53 was detected in 75% of MBs while bcl-2 in 20% of MBs. No correlation was found between the expression of p-53 or bcl-2 and the histological type of tumor. High density of newly formed vessels in association with high expression of Ki-67 and p-53 was detected in all ATRTs. High expression of bcl-2 and p-27 was detected in the majority of ATRTs. Eight patients died (five of the MBs and three of the ATRTs). In all deceased patients, high levels of angiogenesis in association with high expression of both Ki-67 and p-53 was observed.

Conclusions: Increased angiogenesis seems to be associated with high expression of Ki-67, p-53 and p-27. Angiogenesis along with high levels of Ki-67 and p-53, could possibly have a prognostic value for the disease outcome. Study of the above indices at diagnosis could alter or intensify treatment protocols, in order to improve the disease outcome.

Publication

Paediatric oncology

1250

PUBLICATION

Significance of routine bone marrow, CBC and physical examination for early diagnosis and outcome of relapse

M. Baka¹, D. Doganis¹, A. Pourtsidis¹, M. Varvoutsis¹, D. Bouhoutsou¹, V. Duna², H. Kosmidis¹. ¹Children's Hospital Aglaia Kyriakoy, Oncology, Athens, Greece; ²Children's Hospital Aglaia Kyriakoy, Hematology Lab., Athens, Greece

Children with Acute Lymphoblastic Leukemia (ALL) treated according to BFM 90 and 95 in our department after remission induction are routinely evaluated as follows: Bone marrow (BM) four months (mo) from diagnosis (dx) and one mo after therapy completion, complete blood count (CBC) approximately 101 (77 during maintenance and 24 the first 4 years off therapy) and physical exam (PE) approximately 42 (18 monthly during maintenance and 24 while off therapy). Of 50 relapses documented during the study period, 24 – group A – (22 BM, 2 testicular) were diagnosed during routine evaluation (BM 6, CBC 16, PE 2). Group B included 26 symptomatic relapses (fever, echymoses, lymphadenopathy, abdominal pain, headache, vomiting, vision problems), 11 in BM, 6 in central nervous system (CNS), 3 testicular, and combined 6 (BM+CNS 3, BM+testis 3).

Second complete remission (CR) was obtained in 15/24 (62.5%) of group A and in 20/26 (76.9%) of group B. Overall survival (OS) in group A was 29.2% (7/24) for 28–94 mo from relapse (med 53) in CR2 6 and in CR3 1. OS in group B was 38.5% (10/26) for 5–108 mo from relapse (med 25) all in CR2. Of 388 routine BM 6 were positive (0.015%), of almost 20,000 CBC 16 were positive (0.0008%) and of almost 8000 PE 2 were suspicious (0.00025%).

From this retrospective study it is shown that routine evaluation in children with ALL may not depict relapse and therefore may not influence final outcome despite the fact that the number of relapses which we found is significant (24 versus 26).

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PUBLICATION

Preliminary experience with intensity modulated radiotherapy (IMRT) in the management of pediatric brain tumors (PBT)

J. Habrand¹, A. Beaudre², S. Bolle¹, R. El Boustani¹, C. Pichenot², D. Lefkopoulou². ¹Gustave Roussy, Radiation Oncology, Villejuif, France; ²Gustave Roussy, Biophysics Division, Villejuif, France

Introduction: radiotherapy (rt) is a cornerstone in the management of PBTs. Unfortunately its use has been associated with multiple deleterious side-effects esp in young children, that can affect cognitive, pituitary, auditory, visual, functions, and with carcinogenic effects. New technologies, like conformal radiotherapy or IMRT, are considerably promising, but their respective merits rarely quantified.

Patients and methods: Four PBTs in children >6 years, located in the supra tentorial region, were treated using IMRT: superficial cortex (resected ependymoma), deep-sites (craniopharyngioma, optic glioma), and extended complex target (ventricular rt in dysgerminoma). Total dose = 24–55 Gy. 3D virtual simulation was performed based on high definition fused CT/MRI imagings. Target and safety margins were delineated and 3D planning generated (Helios-Cadplan dynamic therapy). Treatment was performed with a Varian machine (52 multileaf collimator). For the purpose of the study, a dosimetric intercomparison with usual conformal rt was made based on dose volume histograms (DVH). Emphasis was put on tight conformation using Conformity Index (CI) to Planning Treatment Volume (PTV), and sparing of critical structures.

Results: Clinical evaluation of the 4 pts showed an acceptable acute tolerance, although close to usual techniques. With a 9–18 months follow-up, all pts have remained with NED, and no complications. Dosimetric evaluation of the IMRT plans, evidenced improved conformation of the 95%, and uniformity of the 100% isodoses to target, especially in case of complex target shapes (ventricular rt: CI: 1.25 vs 2.67). Conversely, hot spots were increased by 5–10%, in most situations. Doses to critical organs were generally lowered with IMRT, although to a moderate extent, and no substantial increase of the integral dose to surrounding structures was recorded.

Conclusion: IMRT is feasible in children and well tolerated. An ultimate conformation to high doses is made possible, especially in case of complex target shapes. No increase of the integral dose to surrounding anatomical structures was observed which tends to indicate that the carcinogenic risk was not impacted negatively.