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Plasma levels of brain natriuretic peptide in children with solid tumors treated by anthracycline chemotherapy

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Background. Plasma levels of circulating natriuretic peptides are elevated in left ventricular (LV) dysfunction and heart failure and have therefore been suggested as noninvasive markers for asymptomatic LV dysfunction. The objective of this study is to evaluate the diagnostic value of plasma brain natriuretic peptide (BNP) as early indicator of cardiac dysfunction in patients treated with anthracycline drugs for childhood solid tumors.

Material and Methods. Thirty-four patients (11 girls) with an age range between 5 and 20 (mean age 12.2 ± 3.44) were included in the study. All patients were clinically asymptomatic and without evidence of residual malignancy. Cardiac function was assessed by ECG, exercise ECG and ecocardiography with both systolic and diastolic functions. BNP was measured before (BNP1) and after (BNP2) exercise testing. Sixteen healthy children (6 girls) with an age range between 6 and 17 (mean age 11.3 ± 3.64) were used as control group.

Results. Mean plasma concentrations of BNP1 and BNP2 were 10,56 ± 10,22 pg/ml and 15,70 ± 14,06 pg/ml, respectively. Mean plasma concentrations of BNP in the control group were 4,09 ± 2,26 pg/ml. BNP1 plasma levels was significantly increased when compared with BNP plasma levels of the control group ($p<0.016$). Although mean BNP2 plasma levels (after exercise testing) were higher than mean BNP1 plasma levels (resting), this increase was not statistically significant ($p>0,05$).

Conclusion. None of the patients developed overt congestive heart failure and all except one had normal shortening fraction but various systolic and diastolic abnormalities were found. Elevated plasma BNP levels can be considered as an early indicator of myocardial damage.

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Elevated blast glutathione levels are associated with decreased sensitivity to prednisolone and to proliferative rate in the in vitro t-cell lymphoblatic leukaemia model CCRFCEM

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Background: Raised blast glutathione (GSH) levels in childhood lymphoblastic leukaemia (ALL) are correlated with high presenting white cell counts (WCC) and an increased risk of relapse but the relationship to drug resistance remains unclear. Using the T lineage leukaemia cell line CCRF-CEM C7, we demonstrate that increased total GSH is associated with resistance to prednisolone and that GSH levels are related to proliferation rates *in vitro*.

Materials and Methods: Total GSH levels were measured using the recycling assay of Tietze. *In vitro* cytotoxicity was assessed using the sulforhodamine B (SRB) colorimetric assay and proliferation was measured using incorporation of ^{3}H thymidine.

Results: Cells grown in the absence of serum had 2-fold higher GSH levels (34.9 v 17.6; $p<0.001$). Higher GSH content resulted in decreased sensitivity to prednisolone ($p<0.05$) and peak GSH concentrations were related to peak rate of incorporation of ^{3}H thymidine. Reducing GSH levels in serum-free culture using buthionine sulfoximine resulted in partial restoration of prednisolone sensitivity ($p<0.05$ at effective dose combinations). Paradoxically, increasing total GSH levels with 10 mM *N*-acetylcysteine under standard (serum-containing) culture conditions, increased the sensitivity to prednisolone.

Conclusions: Our data lends support the concept that redox status is one of the molecular determinants of prednisolone sensitivity in childhood ALL and as such suggests another mechanism that might be exploited therapeutically. Furthermore, microenvironment may also be an important determinant of blast sensitivity to glucocorticoids. The correlation of total GSH with rate of proliferation offers an explanation for its association with high WCC at presentation in children with ALL.

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The impact of infectious events in morbidity and mortality in children with ALL treated with ALL-BFM-90 Protocol

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Leukemia is the most prevalent cancer in children in R. Macedonia; 37% of all children with malignancies presented acute leukemia, and 82% of them were diagnosed as Acute Lymphoblastic Leukemia (ALL). Since the 1997 the German protocols for treatment of children with ALL (ALL-BFM-90, ALL-REZ-BFM-90) have been introduced into University Children's Hospital in Skopje.

Objective of this study was to explore the characteristics of Infective Episodes-IE (as judged by length, timing, leucopoenia, fever, CRP elevation and length of antibiotic treatment) and there impact in morbidity and mortality in children with ALL treated with ALL-BFM-90 protocol.

Methods and Results: We retrospectively evaluated data of 64 patients treated with ALL-BFM-90 protocol between Jan 1997 and Jan 2003 with the minimum follow up of 6 months. The percentage of patients treated by age were <1 year: 1,5%; 1-10 years: 74,5%; 11-16 years: 24%. The rate male:female was 1:0,88. Patients were selected in three risk groups according to the protocol: SR (Standard risk) 23,8%, IR (Intermediate risk) 68,2% and HR (High risk) 7,9%. In SR and IR, Protocol I and Protocol II gave most events (Febrile Neutropenia FN, raised CRP). In HR the second course of cytosine arabinoside gave the highest event frequency. Lungs and upper airways were the more frequent sites of infection. They were detected as a focus of infection in 56,5% of patients (pts). The IE were associated with FN in 86,3% of pts. Mean duration of antibiotic treatment was 15 days during Protocol I, 18 days during Protocol II, 7 and 5 days during Protocol M and Maintence therapy. The follow microorganisms were found as the most frequent causes of the IE: Streptococcus pneumonia, Haemophilus influenza, Staphylococcus aureus, herpes simplex virus, varicella- herpes zoster virus and hepatitis B virus. CRP elevation was seen prior to antibiotic treatment and duration of CRP elevation was 3-4 days shorter than treatment with antibiotics. At the end of antibiotic treatment 40% of pts with IE were current, 59% had association with other complications, two of them died.

Conclusion: Protocol I and Protocol II were the periods with more frequency of IE and FN. Lungs and upper airways were the most frequent focus of infection. Antibiotic treatment combined with therapy against virus infections, prophylaxis of fungal infections and supportive therapy, gave the best results in the treatment of IE.

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Treatment results and prognostic factors in osteosarcoma relapse in children

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Background. Osteosarcoma relapse has a poor prognosis. The aim of the study was to evaluate results of treatment and significance of prognostic factors in pediatric patients with the relapse of osteosarcoma.

Patients and Methods. From 1990 to 2000, we treated 24 patients (pts) with osteosarcoma relapse, median age 15 years (range 9 to 18 years). All patients received aggressive multimodal therapy (chemotherapy/surgery) in previous treatment for classic high-grade nonmetastatic osteosarcoma.

Pulmonary metastases were detected in 21 pts (7 solitary), while 3 pts had local relapse of the disease. Disease-free interval (DFI) was more than 1 year in 9 pts.

Surgery was performed in 18 pts (thoracotomy in 16, amputation in 3 pts). Chemotherapy regimens were administered in all patients: 1. IFO, VP 16 (11 pts); 2. HDMth/IFO VP 16 (7 pts); 3. HDMth/Carbo VP 16 (6 pts).

Results. During 10 to 132 months follow-up period (Me=42 months) overall survival rate in all patients was 41%, DFS was 39%. In the subgroup with pulmonary meta, the overall survival rate was 32%, DFS was 30%. The most significant prognostic factors influencing survival were: presence of solitary pulmonary meta ($p=0.035$), DFI longer than 1 year ($p=0.013$), completeness of resection ($p=0.03$), local relapse of the disease ($p=0.039$) and tumor necrosis over 90% in previous treatment ($p=0.046$).

There were no significant differences in survival in relation to the chemotherapy regimen applied.

Conclusion. The use of aggressive multimodal therapy (surgery/chemotherapy) and evaluation of prognostic factors are necessary for successful treatment in patients with osteosarcoma relapse. Type of chemotherapy regimen should be assessed in randomized multicentric trials.

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Childhood cancer incidence in the Kyrgyz Republic 1985-1999

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Background: The study analyses geographical variations in cancer incidence, trends in survival, health status of long-term survivors of childhood cancer.

Material and methods: The incidence of cancer in children aged less than 15 years between 1985 and 1999. There were 1755 registered cases with new diagnoses of cancer. Male-female proportion 1.3. The study collected data from forms submitted along with histological or cytological findings, and deaths certificates. Histologically verified were 84.7% cases.

The population relative risk was estimated for main tumours in urban and rural areas. Detailed population figures from census have been available at the Kyrgyz National Centre of Statistics. The official estimates have been available for intercensus years. These are based on the census and data on natural population change. The population figures and cancer incidence rates for this report have been provided in for age-groups (0-4, 5-9, 10-14), ethnic groups, for each sex and calendar years of the study period. The crude and age-standardised rates per 1 million have been counted. The classification scheme was used according to the International Classification of Childhood Cancer.

Results: Total age-standardised (ASR) annual incidence rate was 74.8 per million. The most frequent diagnostic groups were leukaemias (ASR-20.8), brain and spinal tumours (7.3), lymphomas (5.4), sympathetic nervous system tumours (4.3), kidney tumours (5.0), soft-tissue sarcomas (4.4), retinoblastomas (3.9), germ cell tumours (3.3), hepatic tumours (1.2), carcinomas and non classified tumours (4.6).

Incidence was significantly higher in the Russians, with an ASR of 114.7 cases per million per year, compared with 77.6 for Uzbeks and 65.6 Kyrgyz children. Assigned risk was higher among Russians in lymphomas (RR= 3.5), neuroblastomas (RR=2.5), than Kyrgyz. Kyrgyz and Uzbeks had an increased RR in tumours of eye and testis, but this was not statistically significant. Part of this apparent increase may have been due to the improved registration of cases in the 1980s, particularly of certain tumour types, and of tumours in older children who may have been treated outside the major paediatric treatment centres. Between the 2 periods, 5-year survival increased from 29% to 47%, with improvements in almost all tumour types. In some tumours (Wilm's, soft tissue sarcoma, leukaemias, lymphomas and others) 5-year survival increased from 45% to 64%. There has been an evidence that with new treatment regimens introduced during the 1980s survival in the 1990s has shown the increased gains.

Conclusions: In Kyrgyzstan, the childhood cancer incidence is low and similar to those reported from Asian developing countries. The data could be used for a wide range of epidemiological and other studies.

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Blood and bone marrow transcripts of tyrosine hydroxylase, dopa decarboxylase, and GD2 synthase in neuroblastoma

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Background: Neuroblastoma is the most common extra cranial tumor in childhood. In spite that multimodal therapy is used the outcome for children with severe neuroblastoma is still poor. The mRNA transcripts of tyrosine hydroxylase (TH) and GD2 synthase (GD2S) are two well-known candidates suitable for identification and monitoring neuroblastoma cells in blood and bone marrow. Dopa decarboxylase (DDC) mRNA also seems to be useful, but has not been systematically investigated. The utility of these transcripts for tracing minimal residual disease should therefore be compared in clinical material.

Material and methods: Real-time reverse transcription (RT)-polymerase chain reaction (PCR) methods were developed to quantify mRNA of TH, DDC and GD2S in blood and bone marrow in children with neuroblastoma. The calibrators used were obtained by amplification of segments of cDNA from the mRNAs of the enzymes, which included the targets.

A total of 229 blood samples from 47 patients and 106 bone marrow samples from 41 patients with neuroblastoma of different stages were analyzed. Cord blood from 52 babies and blood from 26 healthy children, 4 months to 16 years of age, were used as controls.

Results: Blood samples were obtained at diagnosis from 11 children with a stage 1-3 disease. Increased concentrations were found in 4 children regarding TH mRNA, 1 child regarding DDC mRNA and 3 children regarding GD2S mRNA.

Bone marrow samples were also received from 9 patients with stage 1-3 neuroblastoma. Two of them had TH mRNA values above cut off level, one had increased DDC mRNA and five had increased GD2S mRNA.

We received blood samples from 14 children with stage 4 disease at diagnosis. TH mRNA was increased in 13 of the cases, DDC mRNA in 7 cases, and GD2S mRNA was elevated in 10 of the cases.

In 15 children with stage 4 neuroblastoma there were increased bone marrow concentrations of TH mRNA in 14 cases, of DDC mRNA in 12 cases and of GD2S mRNA in 13 cases.

There was a high correlation between TH mRNA and DDC mRNA in blood taking all samples into account. This was not the case between GD2S and TH. The correlations were lower in bone marrow between DDC and TH but still higher than the correlation between GD2S and TH mRNA.

Conclusions: The data indicate that at diagnosis of neuroblastoma sensitivity for detection of stage 4 disease is higher with TH mRNA as compared with both DDC mRNA and GD2 mRNA. It also seems that GD2S is less discriminating of stage 4 from stage 1-3 in the bone marrow since 5 of 9 children with stage 1-3 disease had elevated levels in the bone marrow.

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Severe sepsis in children treated for leukemia in a single unit in Romania

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Purpose: This is a retrospective study aimed to determine the etiology and leading causes of death in severe sepsis related to leukemia in a country with limited financial resources.

Patients and methods: The records of 51 children treated for acute leukemia (ALL 44 pts and AML 7 pts) between 1991-2003 were retrospectively reviewed for severe sepsis. Data concerning clinical, microbiologic and other investigative documentation of infection and outcome were analyzed using the SPSS software programme.

Results: Forty five episodes of severe sepsis were documented in 27 patients. All were under chemotherapy: induction phase (6 episodes), consolidation (34 episodes) and maintenance (5 episodes). Etiology was precised in 58% of episodes: 77% bacterial and 11,5% viral and fungal, respectively. The commonest bacteria was Pseudomonas aeruginosa (23% episodes). Of three fungal infections, 2 were systemic aspergillosis and 1 candidiasis. Severe viral infections were varicella (2 pts) and CMV reactivation (1pt).

All severe septic episodes were determined by neutropenia and its degree of severity significantly correlated with mortality (p 0,05).

The addition of G-CSF in therapy had no significant contribution to reducing fatalities (p 0,58).

Mortality rate by sepsis was 19% and documented bacterial infections were 6: Shigella (2 cases) and Pseudomonas, Acinetobacter, Haemophilus and Staphylococcus aureus (1 case each).

Conclusions: Gram negative infection was the leading cause of morbidity and mortality during treatment of leukemia. Severity of neutropenia was the most important factor predicting fatality.

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Epidemiology of childhood cancer in Bihor and Timis counties during the years 1981-2000

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Background: the objective of this study was the epidemiologic study of childhood cancer in Bihor and Timis counties in order to describe the extend and nature of the cancer burden and to assess the effectiveness of cancer control activities in this part of the country.