Thesis of Ph.D.

Examination of Epidemiology, Possible Prognostic Factors and Treatment of Pediatric Brain Tumors

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Introduction

1. Primary Central Nervous System Tumors of Childhood

A large portion (25-30%) of malignant diseases of childhood is the tumors of the CNS. In several countries incidence of CNS tumors of childhood has increased during the last decade. CNS tumors call for special attention through their high frequency rate and poor prognosis due to their localization and frequent resistance to therapy. Although the Hungarian Pediatric Tumor Registry has been existed since 1971, earlier the incidence of pediatric CNS tumors could only be estimated in Hungary due to registration difficulties.

2. Possible Prognostic Role of Heat Shock Proteins (HSP) in Medulloblastoma

Survival of pediatric patients with medulloblastoma can be predicted based on several factors. Widely known clinical factors of survival are extent of resection, age of patients and presence of metastasis. Beyond these, recently new independent prognostic factors have been discovered, as level of ErbB2 receptor, amplification of c-myc, presence of 17p deletion, level of tirozin kinase C. Contradictory reports were published about prognostic role of apoptosis in medulloblastoma.

Main functions of HSPs are to maintain cell homeostasis in normal condition and to help cell survival under stress. Based on complex functions of HSPs in cells they are capable to influence the process of apoptosis in several ways. HSP 27, HSP 70 and HSP 90 are regarded as inhibitors of apoptosis. There are several reports about the prognostic role of anti-apoptotic HSP 27, 70 and 90 in various adult tumors. Expression and prognostic role of anti-apoptotic HSP 27, 70 and 90 in pediatric brain tumors have not been elucidated yet.

3. Treatment of Pediatric Medulloblastoma/PNET

Treatment of pediatric brain tumors has changed during the last decades. Application of new neurosurgical methods, administration of chemotherapy and the more precise irradiation techniques improved survival and decreased late sequelae in many pediatric malignant brain tumors. A new treatment schedule, called Hungarian Brain Tumor Protocol, was elaborated in 1998 by D. Schuler, which was evaluated in 2004.

4. Incidence of Central Nervous System Metastasis in Childhood Malignancies
Rarely, pediatric non-CNS solid tumors may spread to the CNS by direct invasion or by hematogenous way, both of which will be called CNS metastases in our study. There are only a few comprehensive publications concerning the incidence, the possible treatment and the outcome of pediatric patients with CNS metastasis of a non-CNS tumor.

Aims

My aim was to answer to the following questions:

1. Incidence of the malignant pediatric tumors is well-characterized by the Hungarian Pediatric Tumor Registry for more than 30 years. CNS tumors are exceptions, while these tumor types were regularly treated by neurosurgeons alone. My intention was
   a. to determine the exact incidence of pediatric CNS tumors in Hungary,
   b. to determine the relative frequency of different pediatric CNS tumors based on the new classification of WHO 2000,
   c. to compare the data of CNS tumors in Hungary to that in other countries.

2. I studied
   a. the expression pattern and presence of certain anti-apoptotic HSPs (HSP 27, HSP70 and HSP90) in medulloblastoma,
   b. relation of the expression of HSP 27, HSP70 and HSP90 to clinical factors as age of patient, presence of metastasis, extent of resection and histology,
   c. correlation of expression of certain HSPs (HSP 27, 70, 90) and prognosis of patients.

3. I evaluated the Hungarian Brain Tumor Protocol introduced in 1998 in the aspects of
   a. side effects and tolerability,
   b. outcome of patients,
   c. possible changes in treatment schedule in the future.

4. Development of CNS metastasis of pediatric non-CNS tumors is disregarded in Hungary and all over the world, albeit their presence strongly determines the final outcome of these particular patients. I have examined
   a. different tumor types, what is the rate of the CNS metastasis,
   b. if there is a specific localization of primary disease or the CNS metastasis,
   c. which treatment schedule could be effective,
d. how long is the expected survival time in the presence of CNS metastasis,
e. if there is any difference in epidemiology and survival of patients with CNS metastasis of non-CNS tumors in Hungary and in other countries.

**Patients and methods**

**Patients**

1. Incidence and Relative Frequency of Primary Central Nervous System Tumors of Childhood

Data of children with brain tumor under 15 years of age were analyzed. Patients were classified according to the International Classification of Childhood Cancer (ICCC). For histological classification according to the recommendation of WHO four groups were created: medulloblastoma/PNET, astrocytoma, ependymoma and other tumors (craniopharyngeoma, choroid plexus carcinoma, atypical teratoid/rhabdoid tumors, undifferentiated gliomas).

Data of patients were obtained from the National Pediatric Cancer Registry (N=473), from neurosurgeons (N=354) and general practitioners (N=17).

2. Examination of Expression of HSPs in Medulloblastoma

Samples of 65 patients, who underwent initial surgery for primary medulloblastoma at the National Institute of Neurosurgery between 1988 and 2004 were included in this study.

3. Evaluation of the Hungarian Brain Tumor Protocol

Between 1998 and 2004, 41 patients with medulloblastoma/PNET were enrolled. Mean age of patients was 8.6 years at the time of diagnosis. 17% of patients were younger than 3 years. The tumor localization was infratentorial in 83%, supratentorial in 17% of the cases. At primary diagnosis metastasis was detected by MRI in 9.8% of cases. Histological diagnosis was medulloblastoma in 34 and PNET in 7 cases. All cases of PNET were supratentorial. Patients were treated according to the Hungarian Brain Tumor Protocol. The treatment consisted of surgical removal, irradiation and chemotherapy. During the evaluation of the Hungarian Brain Tumor Protocol the overall (OS) and the progression free (PFS) survival for all patients, low and high risk patients and toxicity of treatment and quality of life (Lansky-Karnofsky score) were considered.
4. Incidence and Survival of Central Nervous System Metastasis in Childhood Malignancies

Medical records of patients treated during a 14-year period, between 1989 and 2002 at the 2nd Department of Pediatrics, Semmelweis University have been analyzed. Only patients with non-CNS solid tumors were included in this study. All solid tumors which showed CNS metastasis confirmed by imaging studies and causing neurologic symptoms were included. The following parameters were recorded: the patients’ age at the time of initial diagnosis and at that of the CNS involvement, primary tumor location and histology, time interval between initial diagnosis and detection of the CNS involvement, primary site of CNS involvement, clinical symptoms, treatment and outcome of CNS involvement.

**Laboratory and statistical methods applied**

**Immunoblot analysis**

For immunoblot analysis fresh frozen tumor sample of 7 patients and a medulloblastoma cell line (Daoy) were examined. Primary antibodies were antihuman mouse monoclonal HSP 27 antibody (sc-13132 Santa Cruz Biotechnology, Inc), antihuman rabbit polyclonal HSP 70 antibody (A0500 Dako Corporation, USA) and antihuman mouse monoclonal HSP 90 antibody (sc-13119, Santa Cruz Biotechnology, Inc).

**Immunohistochemistry**

For immunohistochemical analysis sections of paraffin embedded samples of medulloblastoma were used. The description of primary antibodies can be found at 'Immunoblot analysis’ section.

**Statistical methods**

For statistical evaluation $\chi^2$ test, Kaplan-Meier method, Cox’s F test, log-rank test, t-probe, linear regression analysis were applied.
Results

1. Incidence and Relative Frequency of Primary Central Nervous System Tumors of Childhood

We collected data of 844 patients with brain tumors from 1989 through 2001. The most common histological subgroups of brain tumors were astrocytoma (35.4%) followed by medulloblastoma/PNET (23.2%) and ependymoma (10%). In 1989 the incidence was 3.28 cases per 100,000, by 2001 this was 3.96 cases per 100,000. The average annual rate of increase was 1.3%.

2. Examination of Expression of Anti-apoptotic HSPs in Medulloblastoma

Histology

65 patients’ samples were examined: 32 classical, 31 desmoplastic and 2 large cell medulloblastomas.

Immunoblot analysis

All antibodies mark proteins according to their molecular weight.

Pattern of HSP immunohistochemical staining

In classical medulloblastoma staining pattern of samples could not be connected to any special pattern of tumor cells. Desmoplastic medulloblastoma showed in some cases a special pattern of staining for HSP 70 and 90: perinodular positive staining was observed while central areas remained negative. Apoptosis is increased in the center of the nodules compared to peripheral areas, and the staining pattern of antiapoptotic HSPs is corresponding to this phenomenon. In cases of HSP 27 and HSP 90, but not at HSP 70 there was a difference in the intensity of staining among the samples. Two groups were created based on the cytoplasmic intensity of staining.

The mean rates of positive cells were different in HSP 27, HSP70 and HSP 90. The mean percentage rate of HSP 27 and HSP 90 was less (<30%) than at HSP 70 (>60%). Based on the average expression rate of HSPs for HSP 27 and 90 with a 10% cut off, and for HSP 70 with a 70% cut off two groups were created.

There was significant correlation between intensity of HSP expression and percentage of positive cells in the samples with HSP 27 and HSP90 (p<0.001).

Analysis of clinical factors and correlation with HSPs
Analyzing the correlation of clinical factors (age of patients, extent of resection, presence of metastasis, histology) and percentage rate of HSP positive cells, we did not find significant correlation between any of the groups. Therefore, HSPs are expressed in tumor samples independently of the established clinical factors.

Analysis of Prognosis Based on the Expression Anti-apoptotic HSPs

There was no significant difference in survival depending on the expression of HSPs by log rank test.

3. Evaluation of the Hungarian Brain Tumor Protocol

Characteristics of Treatment

Primary tumor was completely removed in 67% of cases. Neurological deficit after primary surgery occurred in 12% of the cases. The shunt insertion rate for entire treatment course was 15.5%. There was neither treatment-related death nor significant delay nor protocol interruption nor dose reduction due to treatment toxicity.

Survival of Patients

Complete remission was found at the end of chemotherapy in 63%, partial remission in 15% and progressive disease in 22% of the cases. Estimated 5-year overall survival for all patients is 44.1% and the progression free survival for all patients is 41.3% with a median follow-up of 49.1 months.

Survival Based on Extent of Resection

Estimated 5-year survival of patients with partial removal and complete removal were 15.9% and 63.4%. The difference is statistically significant (p=0.001).

Survival Based on Age of Patients

Kaplan Meier 5-year estimated survival was 25% for patients below 3 years, and 45% above 3 years. The difference was statistically significant (p=0.03).
Survival Based on the Presence of Metastasis

Of the 4 patients with primary metastasis, only one is alive, who is in complete remission 70 months after primary diagnosis. Due to the low number of patients with metastatic disease, the survival of patients with localized disease is similar to that of all patients (46%).

Survival Based on Risk Groups of Patients

During evaluation of treatment results 22 patients (54%) were regarded as low risk patients (localized disease, complete removal, older than 3 years), and 19 patients (46%) as high risk patients (lack of any of the above mentioned criteria). The estimated 5-year overall survival is 74.0 % (progression free survival: 66.4%) for low-risk patients and 17.4% (progression free survival: 15.8%) for high-risk patients. The difference of overall survivals of the two groups is statistically significant (p<0.00001).

Based on Quality of Life

For assessment of quality of life of patients Lansky/Karnofsky score was applied. 76% of patients who finished the treatment had 90 to 100 points, 12% had 60 to 80 points and 12% had less then 60 at the end of treatment.

4. Incidence and Survival of Central Nervous System Metastasis in Childhood Malignancies

Between 1989 and 2002, 406 patients were treated with non-CNS solid tumor at our Department. Of these patients, 14 (3.4%) had CNS involvement: 9 patients (2.2%) had hematogenous metastasis and 5 patients (1.2%) had direct tumor extension to the CNS. We observed hematogenous CNS metastasis in soft tissue sarcomas (n=2), in neuroblastoma (n=3), in the tumors of the Ewing’s sarcoma family (n=3) and in non-Hodgkin lymphoma (n=1) with a frequency of 2 to 6 %. Direct tumor extension occurred in soft tissue sarcomas (n=3), in neuroblastoma (n=1) with a similar frequency and in malignant chordoma (n=1).

The accompanying main neurological signs and symptoms were headache (n=8), seizure (n=3), impaired vision (n=2), paraesthesia (n=1), speech problem (n=1), other motor defects (n=3), ataxia (n=1), nausea, vomiting (n=2).

Mean age of patients at the diagnosis of the primary tumor was 7.4 years. Mean time interval between initial diagnosis and the detection of the CNS metastasis was 13.8 months for hematogenous metastases, and 7.0 months for direct tumor extension to the CNS.
Therapy after recognition of CNS metastasis included different treatment modalities (irradiation or chemotherapy alone: n=6, combination of chemotherapy with irradiation or surgery: n=4; combination of surgery, irradiation and chemotherapy: n=2, and triple combination with additional high-dose chemotherapy with stem cell rescue: n=1, no treatment: n=1).

All patients died. The mean duration of survival from diagnosis of CNS involvement was 10.4 months for all metastasis of CNS; 15.9 months in cases of direct tumor extension to the CNS and 7.4 months, when hematogenous metastasis occurred. 1-year survival was 43%. Patient with the longest survival from diagnosis of CNS metastasis, received additional high-dose chemotherapy with stem cell rescue beyond the irradiation, surgical intervention and conventional chemotherapy. Average survival time of patients with CNS involvement was significantly shorter than that of patients without CNS involvement as determined by Kaplan-Meier analysis. Among patients with CNS involvement mean survival time from diagnosis of CNS involvement was significantly longer (p=0.04) with CNS involvement present at the time of initial diagnosis (18.6 months) compared to those with CNS involvement diagnosed later (5.9 months). There was no significant difference in survival of patients with hematogenous CNS metastasis and direct tumor extension to the CNS.

Conclusions

1. Incidence and Relative Frequency of Primary Central Nervous System Tumors of Childhood

In Hungary the relative frequency of CNS tumors among malignant tumors of childhood is higher (26.5%) compared to other countries.

Analyzing the yearly changes of incidence of CNS tumors of childhood in Hungary, we found an increase of incidence between 1989 and 2001 (yearly 1.3%).

At this moment, this is too early to decide, whether this is a real increase or just the consequence of a better diagnostic method (MRI).

2. Examination of Expression of Anti-apoptotic HSPs in Medulloblastoma

In our study anti-apoptotic HSPs were independently expressed from factors as age of patient, extent of resection, M-stage and histological subtype of the patients. Significant correlation was not shown between survival and constitutional anti-apoptotic HSP expression in our patients with medulloblastoma.
Based on special histological staining patterns (increased expression in perinecrotic apparently hypoxic areas and perinodularly in desmoplastic medulloblastoma) and their crucial role to protect ErbB2 receptors from degradation we presume that drugs, which will be able to inhibit expression of HSPs, may be a possible effective therapeutic tool in the future treatment of medulloblastoma.

3. Evaluation of the Hungarian Brain Tumor Protocol

Neurosurgical intervention was successful among our patients compared to international results.

Survival of patients was significantly influenced by the age of patients (lack of radiotherapy below age of 3 years) and the extent of tumor resection, which proved the effectivity of surgical intervention and radiotherapy.

The low overall survival (<50%) is caused by the high rate of high risk patients (almost 50%).

The overall survival of low-risk patients is similar to the international results, which is around 65 to 80% with conventional treatment.

This result may be improved by a more exact stratification of the patients through the molecular examination of tumor cells, leading to relocation of certain patients from low-risk group to high-risk group.

The disappointing overall survival of high-risk patients and the low number of serious life threatening side effects makes possible the intensification of the treatment schedule. Hence we have recently intensified the treatment of high-risk patients by application of high-dose chemotherapy with stem cell rescue.

4. Incidence and Survival of Central Nervous System Metastasis in Childhood Malignancies

CNS metastasis is a rare event of pediatric non-CNS malignancies, and development of that is difficult to predict currently.

Incidence of CNS metastasis of pediatric non-CNS tumors is similar to that in other countries.

The survival of patients with non-CNS solid tumors is significantly shorter in the presence of CNS involvement (both in hematogenous metastasis or direct tumor extension)
when compared to patients without it. There was no effective treatment of CNS involvement in our patients.

Longer survival was achieved in those cases where multi-modal treatment had been applied.

Considering the poor survival and low incidence of non-CNS tumors with CNS involvement it would be necessary to elaborate an effective treatment in an international collaboration to improve the survival of these patients.
Publications related to the theses

Papers:


*Abstracts*

37th Congress of International Society of Pediatric Oncology (SIOP) Vancouver, Canada. September 2005
(oral presentation)

35th Congress of International Society of Pediatric Oncology (SIOP) Cairo, Egypt. 2005.

34rd Meeting of the International Society of Paediatric Oncology, Porto, Portugal 2002

34rd Meeting of the International Society of Paediatric Oncology, Porto, Portugal 2002

32nd. Meeting of International Society of Paediatric Oncology, Amsterdam, Holland, 2000.
Important publications not related to the theses

Papers:


Hauser P, Jeney A, Kralovánszky J: DBM és Busulfan hatásának összehasonlítása csontvelőre és bélnyálkahártyára balb/c egerekben Comoparison of the effect DBM and Busulfan on Bone Marrow and Intestine Mucus Membrane in Balb/c Mice). Magyar Onkológia. 1999;43.43-47


Books:


Dr. Diag. Online diagnosztikai rendszer (https://drdiag.werker.hu/kereso/index.php )

Other papers

Hauser P: Gyermekkori malignus daganatos betegségek a háziorvosi gyakorlatban (Pediatric Malignant Diesase in the Practice of Family Doctors). Hippocrates 2001;3(4):235-238
Abstracts

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*1st prize (Translational Research)*

Garami M, Kertész G, Schuler D, **Hauser P**: Gyermekkori thymus carcinoma sikeres kezelése (Successful Treatment of Adolescent Thymic Carcinoma). Magyar Onkológia. 2005;49

*(oral presentation)*


Földesi E, **Hauser P**, Zsambor C: Worldgame–test in the Field of Pediatric Oncology. Psycho-Oncology. 2003;12(S1-S277): S200