PhD thesis

DIAGNOSTIC, THERAPEUTIC AND BIOCHEMICAL ASPECTS OF CHILDHOOD CANCER MANAGEMENT

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STUDY AIMS

Based on several decades' clinical experience in paediatric cancer care, this study aimed at characterizing and analysing certain, hitherto not fully acknowledged and understood aspects of childhood cancer management, by putting special emphasis on

diagnostic problems due to difficulties in

distinguishing between malignant, and non-malignant processes,
detecting disease associations, and predisposing conditions,
evaluating rare manifestations;

treatment issues such as
recent developments and targeted therapy,
infectious complications, and
acute toxicity and late effects of treatment;and

biochemical topics
in the analysis of paediatric oncological diseases and their chemotherapy.

SUMMARY AND CONCLUSIONS

While cancer in children is relatively uncommon, it remains the leading cause of disease-related mortality among children 1-14 years of age. During 25 years (1975-2000), 594 children with cancer (leukaemia: 262, solid tumours: 332 cases) were treated in our Unit at the Department of Paediatrics, University of Szeged. Our local and Hungarian national data concerning the incidence and survival rates (5-year overall survival) are practically the same as or approach those of the United States and international figures: 130-150 / million children/year, and 75-63%, all groups combined (1985-2000), respectively.
Favourable trends in Hungary are primarily due to the systematization of cancer care, i.e. the establishment in 1971 of the Hungarian Paediatric Oncology Group and the organization of extensive, close collaboration, which resulted in 25 years of progress.
The manifold tasks for Childhood Cancer Centres include the development of expertise in the clinical management of children with cancer, including accurate diagnosis (even in special, rarely encountered conditions) and appropriate treatment, using different modalities,with innovative and targeted therapies, too. Extensive knowledge of the relevant indications, acute toxicities and late side-effects of treatment is also required. Experts in pathology and in various laboratories are indispensable members of this comprehensive collaboration as regards special studies and research topics, coordinated by the paediatric haematologist/oncologist. As many paediatric cancer centres participating in randomized clinical trials and studies are university-affiliated, the importance of specialized training and education at different levels should also be emphasized.
This PhD thesis summarizes studies made with a contribution by the author to the diagnostic, therapeutic and biochemical aspects of childhood cancer management.

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Various clinical entities are classified as rare diseases. They include processes characterized histopathologically by cellular proliferation, and clinically by a progressive course, which pose a number of diagnostic problems necessitating a thorough diagnostic work-up to exclude oncological conditions. As an example, we have analysed pulmonary capillary haemangiomatosis (PCH) in connection with the description of a clinical case.

- **Recognition and differential diagnostics (e.g. excluding a malignant process) and adequate therapy of rare, progressive vascular disorders (such as PCH) with a not completely clear cause/mechanism and natural history, can be of vital importance for the affected children.**

In a group of disease associations, it is not always possible to prove exactly whether one of the conditions serves as a pathological basis for the others, especially if there is a malignant process among the components. In an illustrative case, a chronic persistent erythema nodosum was diagnosed in a girl previously treated for ALL and 12 years later for cerebellar haemangioblastoma. Then, during the several years' history of erythema nodosum, special attention was paid to the possible role of a malignant proliferation, and several attempts were made to elucidate the underlying causal factors. Eventually, the diagnosis of celiac disease could be confirmed and as a result of a gluten-free diet the skin disease resolved, pointing to a causal linkage between the two conditions.

- **Appropriate, early consideration and diagnosis of possible disease associations and/or causal relationships can result in effective management.**

Interrelationships of gastrointestinal disorders with malignant processes is generally accepted. A further instance of a similar association and/or causal link can be demonstrated by analysing our observations concerning the association of adenocarcinoma of the colon and Crohn's disease, as we were not aware of any report on colon carcinoma that developed on the basis of Crohn's disease.

- **Crohn's disease is rare in childhood and its diagnosis is difficult; symptoms of colon carcinoma (which is exceedingly rare in childhood) are similar to those in adults, and to a certain extent to those of Crohn's disease: pain, nausea,loss of weight,and melaena; but its possibility in children is rarely encountered. Accordingly, the tumours tend to be detected in an advanced stage: careful physical examination of children and palpation of the abdomen are therefore indispensable.**

A further type of disease associations (somewhat resembling the previous one in connection with colon cancer) can be characterized, beyond a mere coincidence**,** as a familial inherited condition or consequence of this. Familial adenomatous polyposis (FAP) is characterized by numerous colorectal adenomatous polyps and by extracolonic manifestations, e.g. hepatoblastoma. If these polypase left untreated, colorectal cancer invariably develops. Gardner syndrome refers to the association of colonic polyps with epidermoid skin cysts and benign osteoid tumours. Patients with FAP, and possibly with Gardner syndrome too, have an increased risk of hepatoblastoma; the lifetime risk of developing hepatoblastoma for children of FAP families is approximately 400 times higher than that in the general population. Hepatoblastoma appears to cluster in patients with adenomatous polyposis coli gene mutations.

- **Attention to hepatomegaly (together with genetic and/or ultrasound examination) is particularly important in children of Gardner syndrome families, because the tumour is potentially curable if diagnosed early.**

Diagnostic problems in childhood cancer are due not only to the low prevalence of these entities and to the suspicion or presence of associated conditions (as discussed before), but sometimes also to the almost complete absence of typical symptoms and signs. On hospital admission rare and extreme cases can display a single and unexpected clinical sign only. Most children with Wilms tumour have abdominal distention and/or a palpable abdominal mass accompanied by several, relatively frequent typical symptoms. Erytrocytosis as a presenting sign, however, is not mentioned at all by recent authoritative and reliable sources. In our case, the extreme erytrocytosis found on routine screening (besides extensive differential diagnostic investigations) drew our attention to Wilms tumour.

- **Our observation underlines our obligation to consider an infrequent and unusual sign even if it is the only finding at presentation; a thorough evaluation is needed for children who present with erytrocytosis as this can be due to Wilms tumour, a potentially curable disorder.**

Many of the current cancer treatment modalities available provide only limited effectiveness and are accompanied by significant side-effects. There is a great need for the development of innovative therapies that increase efficacy and decrease morbidity. These therapies involve agents that target specific biologic processes of cancer.

Chronic myeloid leukaemia (CML) is a clonal disorder which appears in less than 5 per cent of all childhood leukaemias. The characteristic genetic abnormality of CML (the cytogenetic marker is the Philadelphia (Ph1) chromosome) is the reciprocal translocation of the long arms of chromosomes 9 and 22, resulting in a bcr/abl fusion gene. The gene product BCR-ABL protein is an active protein tyrosine kinase, which is required for the oncogenic activity. The new therapeutic agent STI571, imatinib, is a competitive inhibitor of tyrosine kinase. There are very few data on experience with imatinib in paediatric practice. In our case of CML refractory to conventional therapy, imatinib treatment produced a complete response: at the end of the fourth year of targeted therapy,the patient lives a normal healthy life in complete clinical, haematologic and molecular-genetic remission 7years after the diagnosis of his primarily refractory, potentially incurable illness.

- **When deciding in favour of imatinib treatment, which is a safe and effective strategy in the treatment of adult type CML (18 moths before the paediatric approval by the FDA), we had to face very hard challenges e.g. professional, ethical, and financial issues, but nevertheless, to the best of our knowledge, there are no data (except for ours) available from the European continent on the childhood use of imatinib, approved as Gleevec for paediatric leukaemia by the US Food and Drug Administration in 2003.**

Highly specific (i.e. targeted) therapy is likely to be much less toxic, and therefore to eventually replace conventional cytotoxic therapy in oncology. Excellent results are currently being obtained with combinations of standard and targeted therapy: various modalities of immunotherapy, including monoclonal antibodies, have been used with promising results. Rituximab is a chimeric mouse/human anti-CD20 antibody, acting on CD20 antigen expressed in different B-cell malignant lymphomas, which we have applied with excellent results for a refractory disease. Combined with an autologous stem cell transplant, the treatment resulted in a complete continuous remission without any clinical, radiological or laboratory signs of malignant lymphoma on follow-up three and a half years after diagnosis.

- **Combined treatment e.g. “sandwitch” type rituximab therapy together with autologous stem cell transplantation, can lead to a complete recovery and to a possible cure rate exceeding even 90 per cent.**

The efficacy of cytotoxic chemotherapy in children with cancer is highly dependent on the tolerability of the regimes, and of their constituents. In consequence of the partial or complete lack of selectivity of the drugs in the protocols, acute or chronic damage to normal cells and/or tissues should be considered in each case. Patients can be rendered immunocompromised as a result of the lesion of cellular elements of the immune system, leading to potentially life-threatening infections. Toxic lesions of vital organs can manifest as a spectrum of functional disturbances, ranging from acute emergency states to mild or severe, transitory or permanent, even lethal, and manifold long-term consequences, late effects influencing the quality of life of the survivors.
Children on cytostatic, immunosuppressive treatment are exposed to the risk of acquiring severe infections, which seriously affect the outcome of the underlying disease: therefore, infections should be diagnosed and treated properly and prevented as appropriate.

Varicella can be dangerous and even lethal in immunosuppressed patients and in children in poor condition due to a malignant disease. We investigated both passive and active modalities of varicella prevention with promising results, arriving at the result that immunocompromised patients should be tested serologically for immunity and, in the event of susceptibility, they should be given varicella-zoster immunoglobulin; then, in remission, active immunization can be performed. Guidelines based on this kind of investigations remained in force till the introduction and widespread use in children of potent antiviral treatment effective against herpes viruses. A recent European protocol (ALL-BFM95) focuses on acyclovir for both prevention and treatment.

- **In principle: varicella vaccine should not be administered to patients who have a cellular immunodeficiency, but persons with impaired humoral immunity may be vaccinated, nevertheless, problems of universal childhood immunization using live-attenuated varicella vaccine require further study.**

Fungal infections are of increasing significance in children with haematologic malignancies owing to the high-dose chemotherapeutic and immunosuppressive treatment and widespread use of broad-spectrum antibiotics, causing concern regarding efficacious prevention and treatment. Major factors predisposing to fungal infections (neutropenia and intensive antibiotic treatment) together with airborne and/or direct contamination, can lead to severe respiratory tract infections caused by Aspergillus species. After Candida species,Aspergillus species constitute the second most common fungal pathogens in the immunocompromised host. Upper airway colonization precedes most cases of invasive infection.
In our case, during the aggressive cytostatic treatment of a malignant process, the fungal infection caused by Aspergillus species responded excellently to the immediate institution of specific
antifungal therapy.

- **This kind of complication, if diagnosed in due time and treated without delay, can resolve relatively rapidly, allowing for the patient an improvement in general condition and a better quality of life, as well as a possibility for continuation of the treatment of the underlying malignancy.**

Variconazole is recently regarded as the initial choice of treatment, but if patients are intolerant or refractory to therapy, effective alternatives include a lipid formulation of amphotericin-B or an echinocandin.

Besides therapeutic trials, the introduction of novel modalities, and efforts to realize and manage complications (e.g. prevention and treatment of infections) affecting immunocompromised patients, therapy-related problems in the management of childhood

cancer involve the recognition, examination and proper handling of early, acute and chronic, late toxicities and side-effects.

In our study concerning the acute toxicity of high-dose Methotrexate treatment, we found renal or hepatic lesion, vomiting or leucopenia in a significant proportion of the patients, but all these symptoms and signs were mild, on average below grade one.

- **First and foremost due to appropriate folinate rescue and careful supportive treatment, HD-MTX toxicity studied by us proved to be relatively low; in other words: HD-MTX therapy proved to be efficient, safe and well tolerated by children with cancer.**

As the proportion and absolute number of childhood cancer survivors increase, the importance of the late effects of the disease and its treatment als increases. Among the manifold sequelae, late nephrotoxicity is characterized by a paucity of available data. A renal function impairment may be due to the malignant process itself or may be secondary to cytotoxic chemotherapy, irradiation, surgical, or even supportive therapy.
In our collaborative study, we evaluated the renal function to assess the late glomerular or tubular changes in 115 children and young adults at least 48 months after completing complex antineoplastic treatment.

- **We found less frequent and less severe late nephrotoxic side-effects than others. Mild to moderate subclinical damage could be identified in many childhood cancer survivors; most patients, however, experienced a spontaneous recovery: due to the routinely applied supportive measures during chemotherapy, the development of significant renal abnormalities was an exception rather than a rule.**
- **Patients at risk should be identified and subjected to check-up programmes to detect and manage kidney-associated late morbidity and thereby preserve the quality of life.**

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Earlier studies detected increased activities of cysteine proteinases (cathepsins) and metalloproteinases in malignant tissue samples. A correlation with metastatic potential was suspected. No data concerning serum enzyme activities in paediatric cancer (ALL and solid tumours) were found in the pertinent literature before our investigations. We studied enzyme activities in the serum of 30 childhood cancer patients: ALL: 22, solid tumour: 8 cases.

- **A significant increase in lysosomal cysteine proteinase, i.e. cathepsin H, was demonstrated in the serum of ALL patients, either in the initial intensive treatment period or in remission or during maintenance treatment.**
- **In the solid tumour group, elevated cathepsin B and H activities were found, in accordance with earlier observations.**
- **From a comparison of the elevated cathepsin enzyme activities with the clinical data on the children (most of them (25/30) undergoing aggressive cytostatic treatment), we can assume a role of cell damage in this phenomenon.**

In a previous pilot study, we tested the plasma D-lactate level as a possible indicator of injuries to the intestinal mucosa. That study included some patients undergoing high-dose MTX treatment. We found a marked fall in plasma D-lactate level, raising the possibility that MTX inhibits the endogenous alpha-oxoaldehyde metabolism: a presumed enzyme blockade could have led to the accumulation of methylglyoxal together with a decreased D-lactate

production. This seemed to be of special interest as impairment of the methylglyoxal metabolism is cytotoxic and glyoxalase I inhibitors exhibit antitumoral activity.
In HD-MTX-treated children, we investigated the plasma D-lactate level, and the inhibition of glyoxalase I by MTX and folates was tested *in vitro*. MTX induced an acute, significant fall in the
D-lactate level. The glyoxalase I activity was inhibited *in vitro* by MTX and folates, too.

- **The present data indicate that MTX inhibits the metabolism of alpha-oxoaldehydes**
  ***in vivo* in leukaemic children, as a likely consequence of glyoxalase I inhibition, suggesting that besides the inhibition of dihydrofolate reductase and thymidylate synthase, the glyoxalase I inhibitory property of MTX may contribute to the anticancer and cytotoxic action of the drug.**
- **As glyoxalase I was inhibited by MTX, folic acid and folinic acid in our in vitro studies, their inhibitory action provides a rationale for the investigation of folic acid and folinic acid in chemotherapy regimes with regard to their activity to alter the resistance to anticancer agent-induced apoptosis.**

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Our studies dealing with the diagnostic, therapeutic and biochemical aspects of childhood cancer management demonstrate certain interconnections between the afore mentioned topics. In paediatric oncology, research is ongoing on several fronts, e.g. to investigate innovative treatment approaches, to study(in order to minimize or prevent) acute and late toxicities, and to analyse and interpret clinical and laboratory biochemical data and mechanisms, which may contribute to a better understanding and, presumably, to a better management of childhood cancer.

LIST OF PUBLICATIONS ON WHICH THE THESIS IS BASED

Full Papers

I **Bartyik K**, Bede O, Tiszlavicz L Onozo B, Virag I ,Turi S.
Pulmonary capillary haemangiomatosis in children and adolescents: report of a new case
and a review of the literature.
Eur J Pediatr. 2004,163:731-7.
IF: 1.369

II **Bartyik K**, Varkonyi A, Kirschner A, Endreffy E, Turi S, Karg E.
Erythema nodosum in association with celiac disease.
Pediatr Dermatol. 2004;21:227-30.
IF: 0.783

III Tiszlavicz L, Kapin M, Varkonyi A, Lorincz A, **Bartyik K**, Varkonyi T.
Adenocarcinoma of the colon developing on the basis of Crohn's disease in childhood.
Eur J Pediatr. 2001;160:168-72.
IF: 1.220

IV Boda D, **Bartyik K**, Szuts P, Turi S.
Active immunization of children exposed to varicella infection in a hospital ward using
live attenuated varicella vaccine give subcutaneously or intracutaneously.
Acta Paediatr Hung. 1986;27:247-252.

V Boda D, **Bartyik K**, Szuts P.
[One-year continuous passive immunization against varicella of children receiving cytostatic and immunosuppressive therapy]
Gyermekgyogyaszat. 1987;38:129-135.

VI **Bartyik K**, Virag I.
[High dose methotrexate treatment. Clinical survey]
Gyermekgyogyaszat. 1992;43:417-23. Hungarian

VII Bardi E, Olah AV, **Bartyik K** Endreffy E, Jenei C, Kappelmayer J, Kiss C.
Late effects on renal glomerular and tubular function in childhood cancer survivors.
Pediatr Blood Cancer. 2004;43:668-73.
IF: 1.362

VIII Laszlo A, Sohar I, **Bartyik K**, Virag I.
Activity of cathepsin B, H, L and a metalloprotinase (MMP7-ase) in the serum of children suffering from acute lmyphoblastic leukaemia (ALL) and tumours.
Clin Chem Enzym Comms. 1991;4:21-5.

IX **Bartyik K**, Turi S, Orosz F, Karg E.
Methotrexate inhibits the glyoxalase system in vivo in children with acute lymphoid leukemia.
Eur J Cancer. 2004;40:2287-92.
IF: 3.302

IF (total): 8.036

Congress Abstracts

X Onozo B, **Bartyik K**, Tiszlavicz L, Csuka D.
Hepatoblastoma in a family with Gardner-syndrome. – Case report.
2003 SIOP XXXV Meeting. Cairo, Egypt.
Med Pediatr Oncol. 2003;41:390. Abstract.
IF: 1.737

XI **Bartyik K**, Onozo B, Turi S.
Paraneoplastic erythrocytosis in Wilms tumor.
2003 SIOP XXXV Meeting. Cairo, Egypt.
Med Pediatr Oncol. 2003;41:391
IF: 1.737.

XII **Bartyik K**, Onozo B.
Good response for a new therapy in a childhood chronic myeloid leukaemia.
2002 SIOP XXXIV Meeting. Porto, Portugal.
Med Pediatr Oncol. 2002;39:414. Abstract.
IF: 1.216

XIII **Bartyik K**, Simon G, Pocsik A, Tornyos S.
Aspergillus infection in a 13-year-old girl suffering from
acute lymphoid leukaemia and histiocytic sarcoma.
2000 SIOP XXXII Meeting. Amsterdam, The Netherlands.
Med Pediatr Oncol. 2000;32:338. Abstract.
IF: 1.301

IF(total): 5.991

Hungarian Paediatric Oncology Group Reports

XIV Schuler D; on behalf of the HPOG.
[The Hungarian Paediatric Oncology Group experience (1994-1997)]
Gyermekgyogyaszat. 1999;50:163-170. Hungarian.

XV Magyarosi E; on behalf of the HPOG
[Hungarian experience in the treatment of childhood acute lymphoblastic leukaemia]
Magy Onkol. 2000;44:255-9. Hungarian.

XVI Kovacs G, Muller J, Borgulya G, Koos R; on behalf of the HPOG
[Treatment results of childhood Hodgkin's lymphoma in Hungary]
Magy Onkol. 2001;45:397-401. Hungarian.

XVII Muller J, Koos R, Garami M Hauser P, Borgulya G, Schuler D, Bardi E, Hunyadi K, Gabor K, Masat P, Bodnar L, Kovacs G; on behalf of the HPOG
[Experiences with Langerhans' cell histiocytosis in children inHungary]
Magy Onkol. 2004;48:289-95. Hungarian.

XVIII Muller J, Kovacs G, Jakab Zs, Renyi I, Galantai I, Bekesi A, Kiss C, Nagy K, Kajtar P, Bartyik K, Masat P, Magyarosy E.
[Treatment results with ALL-BFM-95 protocol in children with
acute lymphoblastic leukaemia in Hungary]
Orv Hetil. 2005;146:75-80. Hungarian

Others

XIX **Bartyik K**.
[The spectrum of neurologic disease in children with systemic cancer]
A review of Antunes NL. Pediatr Neurol. 2001;25:227-35

Gyermekorvos Tovabbkepzes. 2003;2:127-30. Hungarian.

XX Gabor KM, Onozo B, **Bartyik K**, Nagy K.
[Immunotherapy in childhood malignancies: report of two cases]
Hungarian Paediatric Association Southern Hungary Section XXXVII Annual Meeting.
2004, Oroshaza. Abstract. Hungarian.

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