PhD thesis

DIAGNOSTIC, THERAPEUTIC AND BIOCHEMICAL ASPECTS OF CHILDHOOD CANCER MANAGEMENT

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CONTENTS

ABBREVIATIONS

abl	normal homologue (c-abl) of Abelson leukaemia virus transforming gene (v-abl) on human 22q chromosome
AFP	alpha foetoprotein
ALL	acute lymphoid (lymphoblastic) leukaemia
AML	acute myeloid leukaemia
ANC	absolute neutrophil count
BFM	Berlin-Frankfurt-Munster
BM	basement membrane; bone marrow
BCR	breakpoint cluster region gene on 22q chromosome
CHOP	cyclophosphamide hydroxydaunorubicin oncovin prednisone
CML	chronic myeloid leukaemia
CNS	central nervous system
COSS	Cooperative Osteosarcoma Study
DNA	desoxyribonucleic acid
ECM	extracellular matrix
EFS	event-free survival
G(M)CSF	granulocyte (macrophage) colony stimulating factor
HBL	hepatoblastoma
HD	high dose; Hodgkin disease
HPA	Hungarian Paediatric Association
HPOG	Hungarian Paediatric Oncology Group
HR	high-risk
HRCT	high resolution computer tomography
LDH	lactate dehydrogenase
ICCC	International Classification of Childhood Cancer
M-BCR	major breakpoint cluster region
MDR	multidrug resistance
NACI	National Advisory Committee on Immunization
NBL	neuroblastoma
NCI	National Cancer Institute
NHL	non-Hodgkin lymphoma
PAI	plasminogen activator inhibitor
PCH	pulmonary capillary haemangiomatosis
PCV	packed cell volume (=haematocrit)
Ph	Philadelphia chromosome
POG	Paediatric Oncology Group
RBC	red blood cell
RMS	rhabdomyosarcoma
ROS	reactive oxygen species
RT-PCR	reverse transcriptase polymerase chain reaction
SCID	severe combined immunodeficiency
SEER	Surveillance Epidemiology and End Results
SIOP	International Society of Paediatric Oncology
SIOPEL-HRHBL	SIOP Childhood Liver Tumor Strategy Group high-risk hepatoblastoma protocol
STS	soft tissue sarcoma
TIMP	tissue metalloproteinase inhibitor
WT	Wilms tumour
VZIG	varicella-zoster immune globulin
VZV	varicella-zoster virus

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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.

INTRODUCTION

While cancer in children is relatively uncommon, it remains the leading cause of disease-related mortality among children 1-14 years of age. The approximate incidence in Hungary is 15 per 100.000, i.e. yearly about 15 new cases per 100,000 children. The survival rates, however have improved dramatically since the 1960s, when the overall survival 5 years after the diagnosis of paediatric cancer was estimated to be 28%.

The steady decrease in the mortality rates of various childhood malignancies is a tribute to the clinical investigators who for decades have diligently collaborated to conduct clinical trials that have identified improved treatments for children with cancer. The increases in survival rates have continued, with the 5-year survival now exceeding 75%. The survival improvement has been most dramatic for children with ALL.

Incidence and survival data (selected for the sake of comparison and illustration from US, Hungarian national and local sources) are summarized in Table 1, and Figures 1-3.

ICCC group		incidence[1] *	APC[2] *	observed **	5-year survival ***	rate (per cent) ****
	All groups combined	138.4	0.7	74.7	67.6	63.1
I	Leukaemia	42,0				
	acute lymphoblastic	32,7	0,9	81,8	72	71
	acute non-lymphocytic	6,3	1,5	41,1	21	24
II	Lymphomas	14,8				
	Hodgkin	6,0	-1,1	93,6	92	88
	non-Hodgkin	5,4		77,1	70	68
III	CNS neoplasms	29,0	1,1	66,4		48
	medulloblastoma				47	
IV	Sympathetic NS	10,5				
	neuroblastoma	10,2		66	59	51
V	Retinoblastoma	4		94,7		
VI	Renal tumors	8,6				
	Wilms tumor	8,4		90,6	85	76
VII	Hepatic tumors	1,9				
	hepatoblastoma	1,5	3,5	61,0		
VIII	Bone tumors	6,5				
	osteosarcoma	3,5	1,3	66,9	63	47
	Ewing sarcoma	2,4	-0,6	64,7	38	43
IX	Soft tissue sarcoma	10		73,1	54	53
X	Germ cell neoplasms	4,7	1,6	86,7		
XI	Epithelial cancer	5,3		98,2		
XII	Other, non classified	1				
	Total number of patients	(approx)		14000	1049	363

Table 1 Childhood cancer incidence and survival
(selected data from US, Hungarian national and local sources; see text)
(explanatory notes see next page)

(Table 1 explanatory notes)
[1]: average number of annually diagnosed cases per million children
[2]: annual percent change
*: SEER 1975-2000 (102)
**: SEER 1985-1999 (102)
***: HUNGARY 1992-1997 (112)
****: SZEGED 1985-2000

The incidence of Childhood cancer exhibits a steady change, which is significantly different from zero (P<.05), and positive, i.e. an increase in several groups, subgroups and in all groups combined, (as an exception, negative changes,i.e. a decreasing incidence, are observed in Hodgkin lymphoma and Ewing sarcoma), accompanied by a simultaneous decrease in mortality, i.e. an improving survival. The Hungarian national and our local figures are approaching the US data. Distribution of various subgroups and entities of childhood cancer correspond to those reported from the USA, except markedly higher proportion (27 % versus 21 %) of CNS tumours (102, 112). During 25 years (1975-2000), 594 children with cancer(leukaemia: 262, solid tumors: 332 cases) were treated in our Unit at the Department of Paediatrics, University of Szeged.

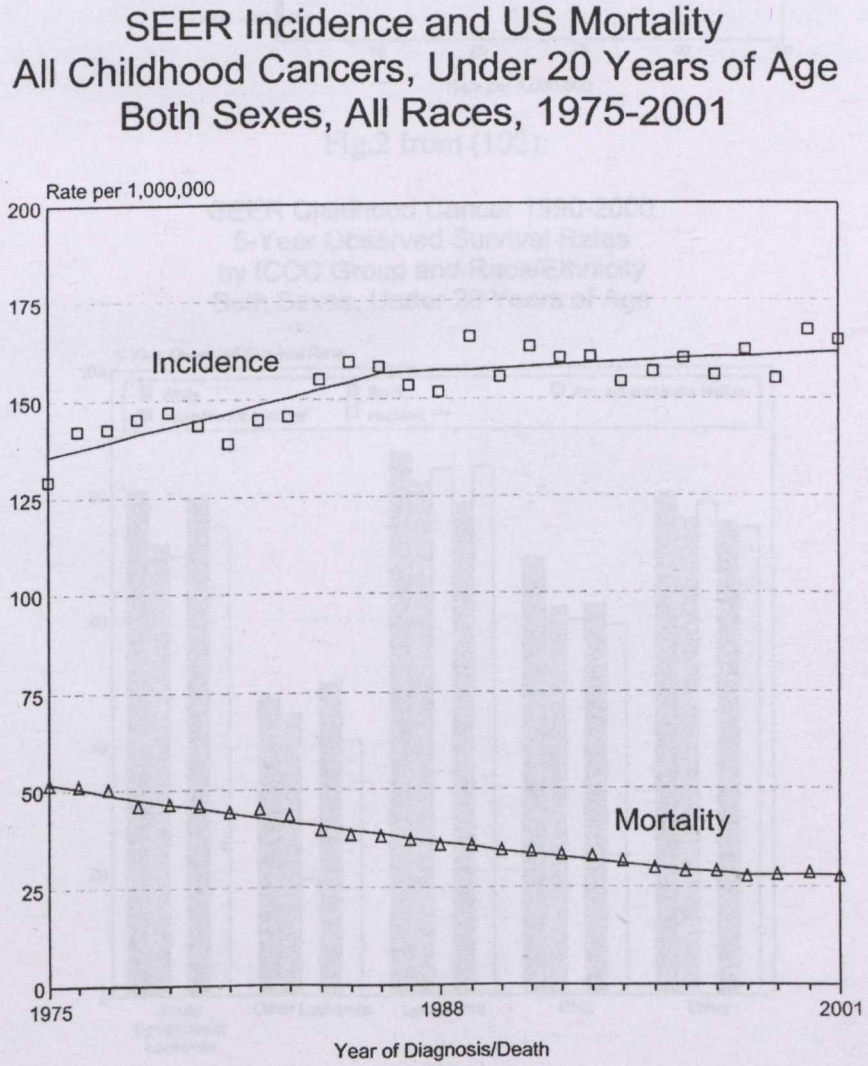

Fig 1. from (102).

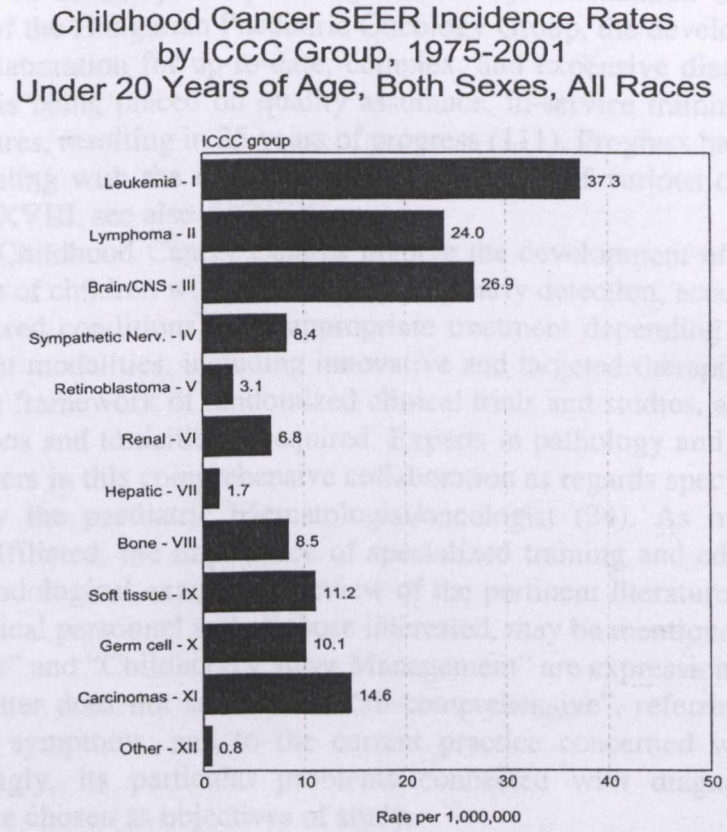

Fig.2 from (102).

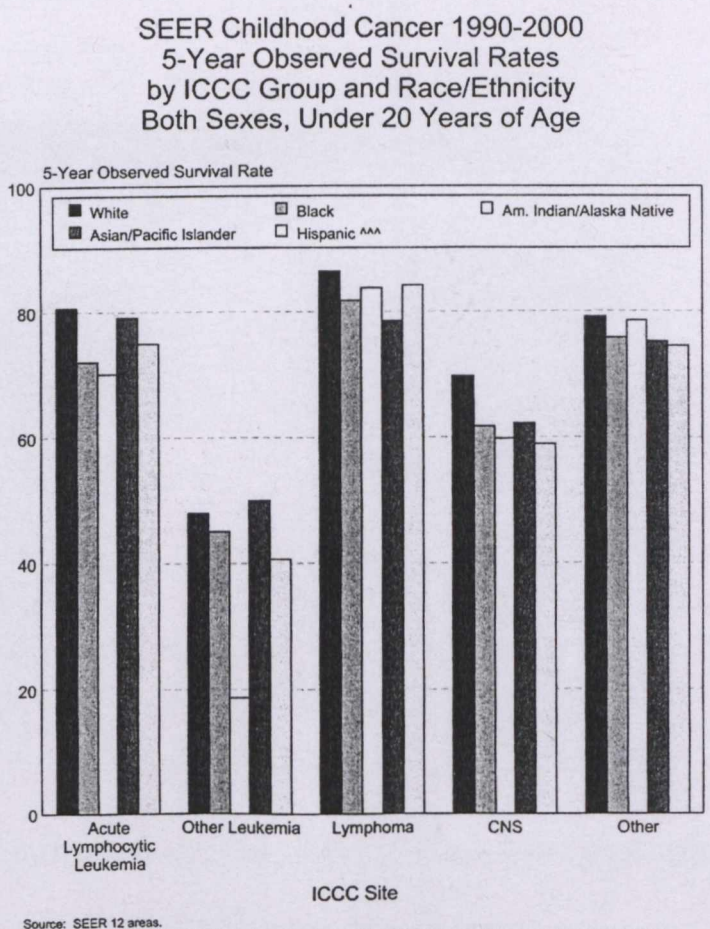

Fig.3 from (102).

The favourable trends in Hungary are primarily due to systematization of cancer care i.e. the establishment in 1971 of the Hungarian Paediatric Oncology Group, the development its Data Centre, the organization of collaboration for up-to-date, complex, and expensive diagnostic and therapeutic modalities; the emphasis being placed on quality assurance, in-service training, cost considerations, and psychosocial measures, resulting in 25 years of progress (111). Progress has been demonstrated in HPOG publications dealing with the results of the management of various childhood malignancies (XIV, XV, XVI, XVII, XVIII, see also ANNEX).

Manifold tasks for the Childhood Cancer Centres involve the development of collective expertise in the clinical management of children with cancer, including early detection, accurate diagnosis (even in special, rarely encountered conditions), and appropriate treatment depending on a multidisciplinary approach, using different modalities, including innovative and targeted therapies. For these therapies, conducted mainly in the framework of randomized clinical trials and studies, an extensive knowledge of the relevant indications and toxicities is required. Experts in pathology and in various laboratories are indispensable members in this comprehensive collaboration as regards special studies and research topics, coordinated by the paediatric haematologist/oncologist (24). As many paediatric cancer centres are university-affiliated, the importance of specialized training and education should also be emphasized. As a methodological example, a review of the pertinent literature aiming at information of the medical, paramedical personnel and all those interested, may be mentioned (XIX).

"Childhood Cancer Care" and "Childhood Cancer Management" are expressions used interchangeably in the literature. The latter does not seem to be "all-comprehensive", referring mostly to particular diseases, conditions or symptoms, and to the current practice concerned with the diagnosis and therapy (96). Accordingly, its particular problems connected with diagnostic, therapeutic and biochemical aspects were chosen as objectives of study.

Various clinical entities discussed in this chapter 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.

1.1 Pulmonary capillary haemangiomatosis in children and adolescents

Case report

A 14-year-old boy with dyspnoea and exercise intolerance was admitted. Four years earlier, he had been hospitalized after recurrent respiratory tract infections. The physical and laboratory examinations, allergological, microbiological, immunological and haematological (including bone marrow) investigations did not reveal any pathology, but the chest X-ray film and echocardiography demonstrated a pronounced cardiomegaly due to pericardial effusion. Treatment included continuous pericardial drainage resulting in clinical and radiological recovery, and a symptom-free period of three years. Thrombocytopenic purpura, epistaxis then ensued requiring prednisolone treatment for two months. For two more months the boy remained symptome-free.
The clinical course was characterized by mild respiratory symptoms and signs. The chest X-ray film and CT scan (Fig4, Fig5) indicated a contrast-enhancing process in the upper mediastinum, and bilateral focal, patchy infiltrates.

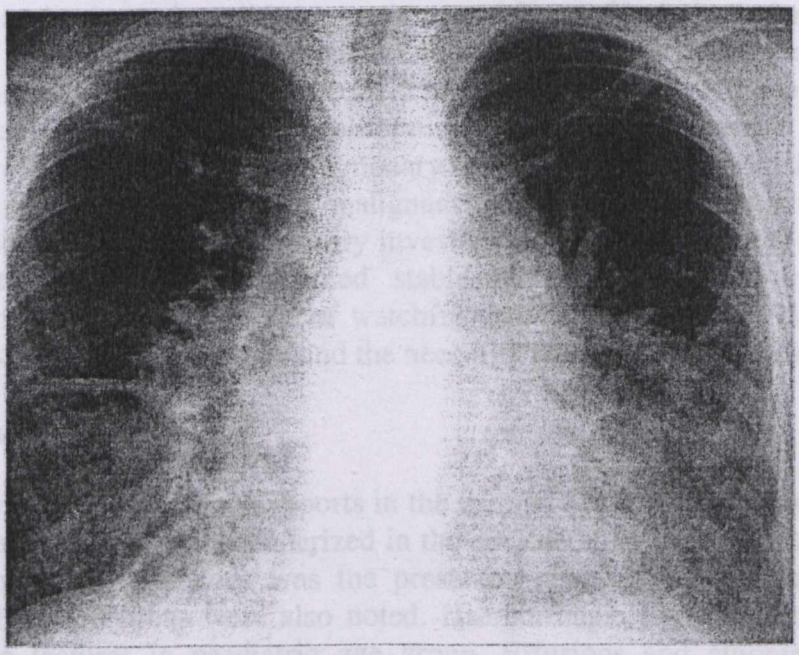

Fig. 4. Chest X-ray showing mediastinal widening and bilateral interstitial infiltrates

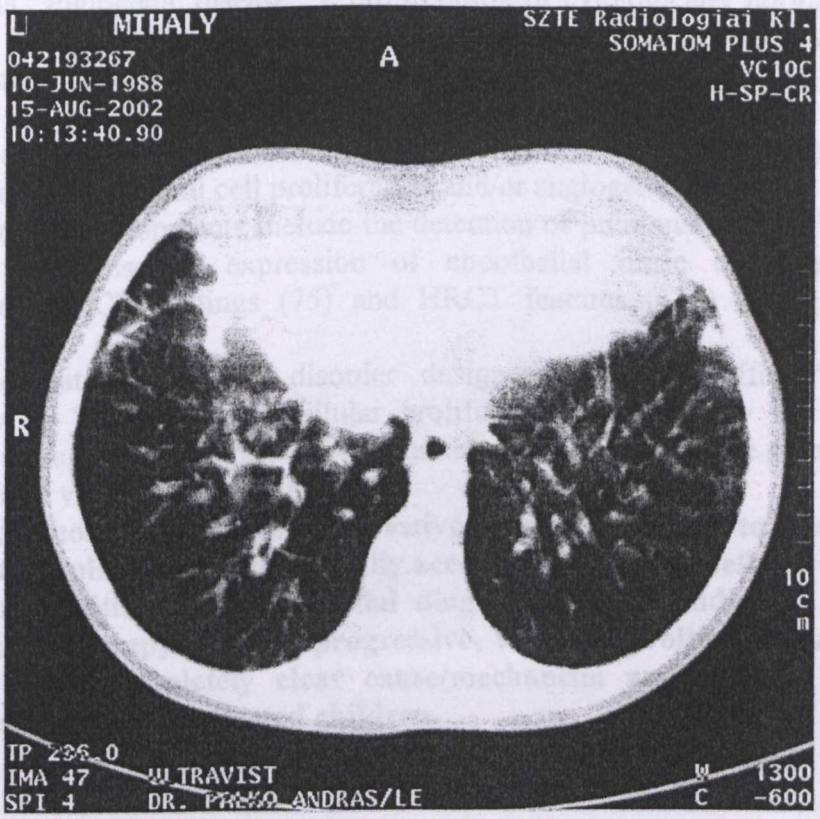

Fig 5. CT-scan (see text)

Pulmonary function studies revealed a slightly restrictive pattern. A mediastinal CT-guided percutaneous core needle biopsy was performed: histological examination showed only reactive inflammation. As the patient did not improve and X-ray films showed no regression either, an open lung biopsy was performed. Histological examination revealed proliferation of the capillary vessels with haemosiderosis and slight fibrosis. The overexpression of the p53 oncoprotein suggested a process of neoplastic origin but the proliferation rate (pKi67) was low.
These features are characteristic of pulmonary capillary haemangiomatosis.
In order to detect any systemic or malignant disorder, various imaging procedures and extensive haematological and chemical laboratory investigations were performed, all yealding negative results. As the patient's condition remained stable thereafter, without any evidence of pulmonary hypertension, a decision in favour of watchful waiting was taken. A close follow up is required to consider possible further treatment and the necessity of lung transplantation.

Discussion and conclusions

Previous reviews (3, 4) and case reports in the literature revealed 14 patients aged up to 19 years with PCH. The main features are summarized in the enclosed full paper (see ANNEX). In 9 of 15 patients (including our case) dyspnoea was the presenting symptom, haemoptysis, febrile conditions and respiratory tract infections were also noted. Haemorrhagic signs and thrombocytopenia seem to be characteristic features in paediatric age group. Effusions and clubbing also occurred. "In vivo" diagnosis could be established in 7 cases, all these were alive at the time of publication. Some initial diagnoses were "inappropriate" even "misleading"; this fact points to the difficulties connected with the clinical, imaging, and also pathological-histological evaluation. Eight patient's PCH was diagnosed on autopsy. Their median survival was three years.
Interferon alpha-2a treatment (in 3 of the 15 cases) proved effective, nevertheless, lung transplantation may be regarded as the treatment of choice. These 15 paediatric/adolescent cases were diagnosed during a 25 year period, thus, in spite of growing diagnostic alertness, PCH remains an extremely rare

disease.In this "angiogenic disease", a proliferation of cytologically normal capillaries can be found (72). PCH is not a neoplastic process, the endothelial cells are not atypical, mitosis is rare, the p53 expression is usually negative and the Ki67 proliferation is slight (8). Vascular proliferative or angiomatous diseases – among them PCH – depend on angiogenesis, and this may have therapeutic implications (39). In contrast to those in adults, only a few studies have been performed in children with inhibitors of endothelial cell proliferation and/or angiogenesis (59).
Recent important developments include the detection of pulmonary arterial hypertensive remodelling by reduced microvascular expression of endothelial nitric oxide synthase (NOS-III) (65). Multidetector row CT findings (75) and HRCT features (115) are also of peculiar diagnostic importance.
One can speculate whether a disorder designated by the suffix "-omatosis", characterized histologically by "uncontrolled" cellular proliferation, accosionally by oncogene-expression, and clinically by a progressive course, can – or rather: should – reliably be separated – from theoretical or practical point of view – from cancer.

- **The diagnosis of this rare, progressive disorder may lead to effective therapy: at present lung transplantation is universally accepted as the final definitive treatment for PCH.**
- **The recognition and differential diagnosis (e.g. excluding a malignant process), and adequate therapy of rare, progressive, vascular proliferative disorders (such as PCH) with a not completely clear cause/mechanism and natural history, can be of vital importance for the affected children.**

1.2 Erythema nodosum associated with celiac disease in a teenage girl with cured acute lymphoblastic leukaemia and haemangioblastoma

Background

In a group of disease-associations, it is not always possible to prove exactly which of the conditions serves as a pathological basis for the other(s), especially if there is a malignant process among the components. In an illustrative case – presented below – a chronic persistent erythema nodosum was diagnosed (II). Erythema nodosum is the most common form of panniculitis characterized by inflammation of the fat septa. Its pathogenesis is believed to involve an allergic or immune complex-mediated reaction to a wide variety of antigens.The most prevalent triggering factors are infections, sarcoidosis, lymphoma, Crohn disease, adverse drug reactions, and pregnancy. However,
in up to 50% of the cases the etiology remains unknown (27). Erythema nodosum generally resolves in 5-8 weeks; however, if the antigenic stimulus persists, the disease may last considerably longer requiring identification and elimination of the underlying disease, accordingly, an exhaustive investigation.

Case report

A 16-year-old girl was referred for consultation because of multiple painful nodules
on the legs (Fig. 6).

The disease had begun four years earlier with recurrent crops of the skin lesions not accompanied by systemic symptoms. In approximately 1 year the disease became persistent.
At the age of two years the patient had been treated for acute lymphoblastic leukaemia. She recovered after two years of therapy and has subsequently remained free of disease. At the age of 14 years, a computed tomography scan performed because of severe headaches revealed a 4 cm tumor in the vermis of the cerebellum.It was removed surgically; the histologic diagnosis was haemangioblastoma capillare (WHO grade I). In the follow-up period recurrence did not occur. Removal of the tumor did not influence the activity of the skin disease.

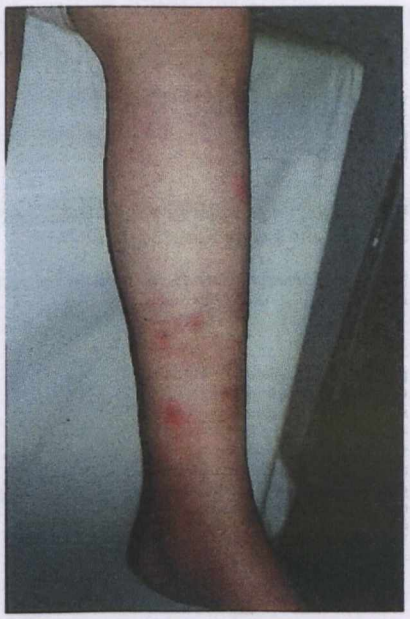

Fig 6. Multiple bright red to brown subcutaneous nodules are present on the extensor surface below the right knee. Similar lesions were found on the left leg.

At the age of 15 years, the histologic analysis of a biopsy specimen from one of the skin lesions confirmed the clinical diagnosis of erythema nodosum.
During the 4-year history of the disease, special attention was paid to the possible role of a malignant proliferation and several attempts were made to elucidate the underlying causal factor(s). All the results of extensive laboratory investigations including chemical, immunological and microbiological tests were normal or negative except for a low serum iron level on several occasions. The latter suggested the possibility of a malabsorption syndrome. The xylose, tissue transglutaminase and endomysium-antibody tests' results indicated celiac disease. The histology of the biopsy specimen from the small intestine demonstrated a subtotal villous atrophy and other typical signs confirming the diagnosis of celiac disease. The girl was put on a strict gluten-free diet. Within a month, the development of new nodules ceased and the old leions resolved. After a three months symptom-free period, she was unintentionally exposed to gluten, followed by the appearance of erythematous, infiltrated nodules on her legs, which regressed on the gluten-free diet, pointing to a causal linkage between the two conditions. Thereafter, the girl remained free of skin lesions.

Discussion and conclusions

Diverse skin lesions have been observed in association with celiac disease, their pathomechanism is considered to be mediated by the increased intestinal permeability to exogenous antigens and the induction of hypersensitivity reactions or the formation of immune complexes. A causal linkage between coeliac disease and erythema nodosum was first observed in 1991 (32). Erythema nodosum is a common finding in sarcoidosis, which frequently coincides with coeliac disease (79). It suggests that other cases earlier attributed to sarcoidosis may actually have been due to coexistent coeliac disease.
The protracted course of skin lesions in our patient was first presumed to be related to the malignant proliferations. Erythema modosum has often been found associated with Hodgkin disease, non-Hodgkin lymphoma and leukaemia; its association with solid tumors is less common (7). The skin lesions related to lymphoproliferative disorders usually have a long duration, and they may precede the diagnosis of malignancies by months (116). To date, however, neither the ALL nor the haemangioblastoma recurred in our patient and the removal of the tumor had no influence on the activity of her skin lesions, therefore this latter kind of pathogenetical linkage – although theoretically can not be excluded with certainty – does not seem probable.

On the other hand, the increased risk of malignant diseases related to celiac disease, and especially that of T-cell lymphomas, is well documented (15). Moreover, the incidence of the malignant consequences can be reduced by the early introduction of a strict gluten-free diet (22).

- **In the present patient, the skin symptoms resolved on a strict gluten-free diet and they recurred after an exposition to the protein. Thus celiac disease can be a triggering factor for erythema nodosum.**
- **In the chronic forms of the skin lesions, serologic testing for this specific enteropathy may be justified**
- **In principle: appropriate, early consideration and diagnosis of possible disease-associations and/or causal relationships can result in effective management.**

1.3 Adenocarcinoma of the colon developing on the basis of Crohn's disease in childhood

Background

As mentioned in connection with the previous case (1.2), interrelationship 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 following observation which may lead to remarkable conclusions contributing to better management of the problems involved (III).
Colorectal carcinoma is distinctly rare in childhood, its relative frequency among types, subgroups of paediatric tumours does not reach 1% (113). The literature reports fewer than 100 cases of malignant tumours of the digestive system developing under the age of 15 years. Nevertheless adenocarcinoma accounts for the most frequent malignancies affecting the digestive tract of young subjects. Ten percent of children affected have some predisposing condition: polyposis, ulcerative colitis (19).
In adults, cases of colorectal adenocarcinoma associated with underlying Crohn's disease have been reported and the topic has been intensively investigated (94). We are not aware of any report on colon carcinoma in childhood that developed on the basis of Crohn's disease.

Case report

Our patient, a 15-year-old girl underwent an appendectomy at the age of 10 years. Histological examination and reexamination revealed ulcerophlegmonous appendicitis without any evidence of Crohn's disease. Shortly thereafter she was hospitalized because of abdominal pain and diarrhoea. Iron deficiency anaemia and lactose malabsorption were detected. Three years later she was hospitalized again because of clinical symptoms of malabsorption associated with somatic retardation. During the next two years, diarrhoea and anaemia together with a lag in physical development have been persistent.
After extensive clinical and laboratory, imaging and endoscopic work-up, a space-occupying lesion in the oral part of the sigmoid with multiple regional lymph node involvement was ascertained by computer tomography. The patient underwent surgery, and the resection extended 10 cm in the oral and aboral parts of the lesion proper and included a wedgeshaped mesocolic tissue.

Microscopically the tumor proved to be a moderately well-differentiated adenocarcinoma (Fig 7.), and 4 lymph nodes with metastatic cancer were detected.

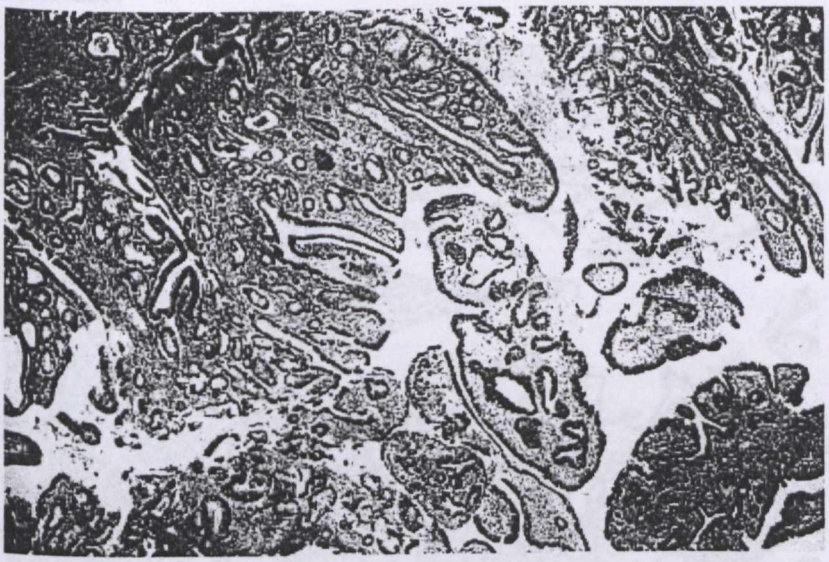

Fig 7. Histologically,the tumour is a well-differentiated Adenocarcinoma (H&E,x112)

The p53 oncoprotein was detected in almost all tumour cell nuclei (Fig 8.), and it was also found in parts of the intestine free of tumour.

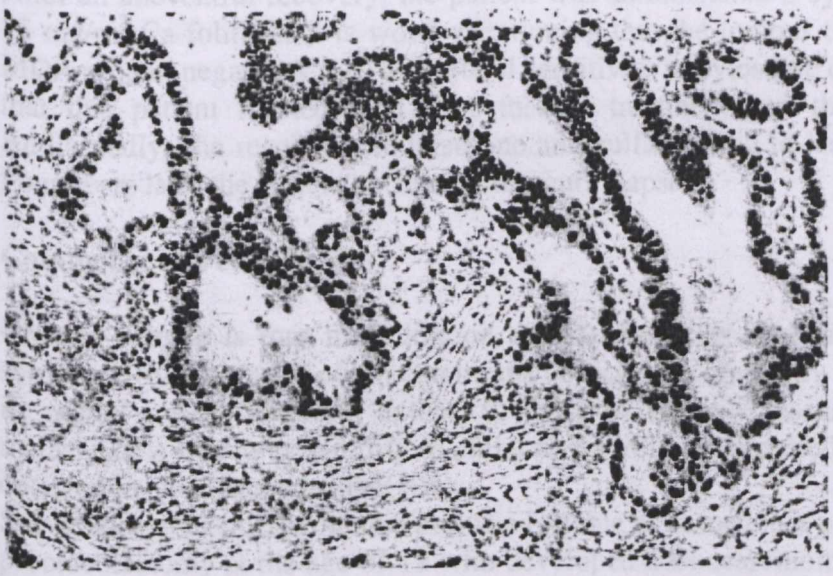

Fig 8. Immunohistochemistry:p53 overexpression in the nuclei of the tumour cells (p53-ABC,x112)

Aphthoid ulceration, fissuration, oedema and inflammatory infiltration were found in the lamina propria of the tumour-free mucosa (Fig 9.); hypoplasia was noted at the mucosal/submucosal interface.

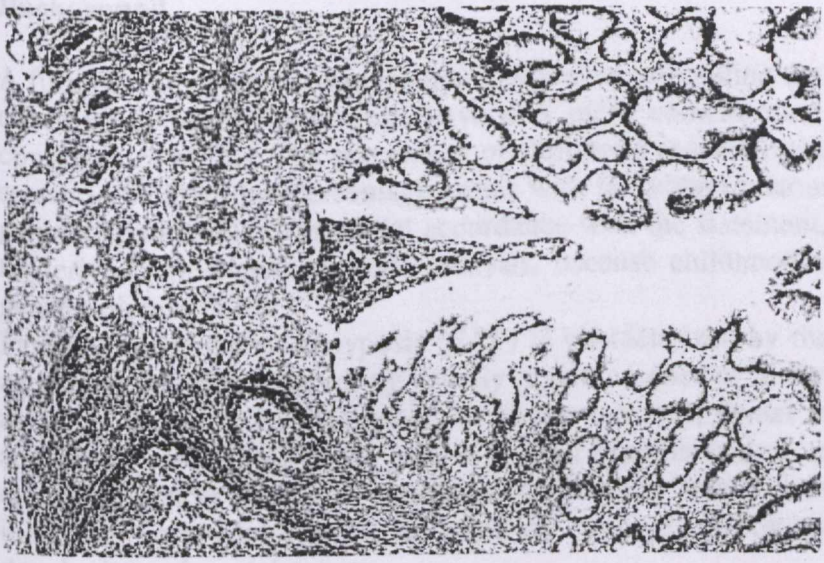

Fig 9. Aphtoid ulceration with volcanic exudates and granulomatous inflammation with lymphoid follicles in a distant part of bowel (H&E,x224)

The final diagnosis was adenocarcinoma of the large bowel with lymph mode metastases and Crohn's disease of the colon.
After an uneventful recovery, the patient was administered 6 cycles of 500 mg/m^2 5-fluorouracil and 25 mg/m^2 Ca-folinate. It is worth mentioning that the tumour tissue immunohistochemistry revealed MDR protein negativity (i.e. theoretical sensitivity to cytostatic drugs). This was supported by the fact that this patient reacted well to cytostatic treatment and there was no haematogenous spread. Additionally, she received prednisolone and sulfasalazine to handle her underlying Crohn's disease. Four years later the patient suffered a tumour relapse.

Comments and conclusions

Crohn's disease is rare in childhood and its diagnosis is difficult: Melaena is the most important symptom. 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,however its possibility in children is rarely encountered. Accordingly, the tumours tend to be detected in an advanced stage(104).
Survillance after operation also seems advisable as shown in a patient with Crohn's disease treated by proctocolectomy at the age of 12 who developed adenocarcinoma 25 years later at the site of ileoanal anastomosis (64).

- **This first reported case of p53 positive colon carcinoma in childhood that developed on the basis of Crohn's disease reveals that prolonged chronic inflammation (Crohn's disease) can be associated with loss of the normal p53 function, which can contribute to the development of dysplasia or carcinoma.**
- **Careful physical examination of children and palpation of the abdomen are therefore indispensable because the tumour can be palpable, and abdominal distention can be observed at the time of the first examination in more than half of the cases.**

1.4 Hepatoblastoma in a family with Gardner syndrome: state of art and brief case report

Background

A further type of disease-association (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. The percentage of childhood cancers caused by a clearly inherited predisposition seems to be low: that percentage varies with individual tumour types and is a composite of different genetic factors.This is in partial accordance with the statement, that real understanding is more likely to derive from a genome wide analysis, because childhood cancer, like every cancer is at heart a genetic disease (130).
Familial adenomatous polyposis (FAP) is characterized by the development of numerous colorectal adenomatous polyps appearing usually in adolescence or in the third decade of life, and by a variable range of extracolonic manifestations (brain tumours, dental anomalies, desmoid tumours, duodenal adenomas, epidermoid cysts, gastric polyps, hepatoblastoma, osteomas). These polyps left untreated, colorectal cancer invariably develops by the early forties at latest. Annual colonoscopy is therefore indicated from adolescence, followed by prophylactic (surgical) therapy to eliminate the risk of developing colorectal cancer.
The adenomatous polyposi coli (APC) gene, mapped to 5q21, consists of 8535 bp spanning 21 exons, encodes a 2843-amino acid protein, a tumor suppressor. APC mutations almost always result in a truncated protein product with abnormal function. Germline (inherited) mutations in the APC gene – its exon 15 comprises > 75% of the coding sequences and is the most common target for mutations occurring mostly at codons 1061 and 1309: apart from these peaks they are spread uniformly between codons 200 and 1600 – are responsible for FAP, while somatic mutations in APC occur in about 80% of sporadic colorectal tumours (36, 91, 133).
Gardner syndrome refers to the association of colonic polyps with epidermoid skin cysts and benign osteoid tumours of the mandible and leg bones. Although rare in the general population, patients with FAP (and possibly with Gardner syndrome, too) have a significantly increased risk of hepatoblastoma. The lifetime risk of developing hepatoblastoma for children of FAP families is approximately 400 times higher: one case per 250 persons, compared with 1 per 100 000 in the general population
Hepatoblastoma appears to cluster in patients with Adenomatous polyposis coli gene mutations at the 5' end of the gene (between codons 457 and 1309).
Far-reaching delineation of the details – as shown recently (86) – would go beyond the scope of this work.
Since the original description "Hepatoblastoma and polyosis coli' in 1982 (61), less than 40 childhood cases of this association have been published, either in patients suffering from FAP or in members of FAP families.

Case report

A one-year-old boy presented with rapidly increasing hepatomegaly. His mother and mothernal grandmother are diagnosed and treated for Gardner syndrome.
Abdominal ultrasound and computer tomography found a liver tumour occupying several segments, thus it was regarded as inoperable. A fine needle biopsy specimen was diagnosed histologically as hepatoblastoma, immunohistochemistry revealed CKA1/AE3, pKi67 and AFP positive pattern. Treatment was initiated according SIOPEL-3 HRHBL protocol and had been performed for three months. The residual tumor could be completely resected, with no evidence of regional or distant spreading. Four months later local relapse occurred and individual chemotherapy regimen was instituted. At the same time, efforts for a liver transplant were started. The mutation of APC gene was identified in the patient, in his healthy brother, and mother, respectively, with DNA-sequencing. The patient survived only 15 months from diagnosis.

Comments and conclusions

Intensive screening of FAP kindreds for extracolonic manifestations facilitates early detection. Hepatoblastoma occurs primarily in boys in FAP kindreds, and is associated with germline APC mutation, however, the site of mutation cannot be used to predict the occurrence. Systematic ultrasound examination of the abdomen should be performed in young sibling of patients with FAP who have an enlarged liver on physical examination (18).
Pigmented ocular fundus and osteomatous jaw lesions are also important clinical signs (68).
Recent data emphasize the importance of the relationship described here, and that of diagnostic testing and cancer screening in affected patients, and the spectrum of APC mutations, as well (47,108).

- **Association between hepatoblastoma and familial adenomatous polyposis with the mutation of the APC gene has been confirmed.**
- **Our case represents more specifically a rare and not generally known occurrence of childhood hepatoblastoma in a family with Gardner syndrome.**
- **Follow up of hepatoblastoma survivors is important because of the increased risk of developing adenomatous polyposis.**

*

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.

1.5 Paraneoplastic erythrocytosis in Wilms tumour: state of and case report

Background

Wilms tumor (nephroblastoma) is the most common primary renal neoplasm in children occurring usually at the age of 3-5 years. Its relative frequency among childhood malignancies is around 6-7%. Developments in the treatment – e.g. chemotherapy regimen, surgical technique – and in the supportive care led to a dramatic improvement of prognosis in the last decades with a cure rate above 80 percent. At the Department of Paediatrics, Szeged University 594 cancer cases were treated over a period of 25 years from 1975 to 2000. Wilms tumour was diagnosed in 37 cases, with a relative frequency of 6.*2%. 5-year event-free survival was 73%, 8 patients (22%) died, 2 (5%) were lost for follow-up.
Most children with Wilms tumor have abdominal distension and/or a palpable abdominal mass accompanied by several, relatively frequent clinical symptoms: abdominal pain, anorexia, vomiting, and malaise (in about 30-40% of cases); and hypertension or haematuria can also occur. Erythrocytosis as a presenting sign, however, is not mentioned at all by recent authoritative and reliable sources (12, 58, 69).Simultaneous occurrence of polycythaemia and nephroblastoma was detected a long while ago in 1963 (82). Both Wilms tumor in adults and polycythaemia in Wilms tumor are rare, nevertheless, a number of cases were described in (young) adults (20, 30, 103, 118).
In our case, the extreme erythrocytosis (besides extensive differential diagnostic investigations) drew our attention to Wilms tumour.

Case report

A 7-year old girl without any complaints and symptoms was admitted because of an extreme erythrocytosis found on complete blood count analysis on the occasion of a routine screening, with normal findings on physical examination. CBC – besides normal values of leukocytes and platelets – showed extremely high red blood cell values; RBC: 7.99×10^{12}/L, PCV: 0.66, haemoglobin: 208 g/L.

All the other routine laboratory data (including reticulocytes, urine analysis, electrolytes, renal and hepatic function, as well as coagulation studies) were normal.
Differential diagnostic investigations can be summarized as follows. Relative or spurious polycythaemia could be excluded at first sight: there were no data pointing to decreased plasma volume (due to reduced fluid intake or marked loss of body fluids). As to absolute polycythaemia, both primary congenital familial disease and polycythaemia vera were unlikely because familial, clinical and/or laboratory signs were absent. The possibility of conditions leading to the third subgroup of absolute polycythaemia – namely the secondary forms – were thoroughly investigated. Congenital factors like abnormal high-affinity haemoglobin variants or methaemoglobinaemia were exluded based on normal haemoglobin electrophoresis, $P_{50}O_2$ and methaemoglobin findings. Besides complete lack of clinical symptoms and signs, normal imaging and laboratory test results (among them arterial O_2 saturation above 92 per cent) did not support the presence of cardiopulmonary factors. For the evidently healthy child in a completely good condition there remained
the possibility of aberrant erythropoietin production due to a renal tumor.
Careful physical examination did not find any abdominal mass on palpation. Abdominal ultrasound and CT imaging revealed a relatively well circumscribed lesion of echodense, respectively of calcific density character, approximately 3 cm of diameter, at the border of the middle and lower third part in the right kidney. Based on the available findings, a decision of surgical removal was taken. On intraoperative histological examination the lesion proved to be Wilms tumor, which could be completely removed together with the right kidney. There were no signs of local extension, and histological examination found a sharp separation from the intact renal tissue. A diagnosis of well-differentiated monophasic-epithelial Wilms tumor was established, belonging to the prognostic group of favourable histology.
No further therapy was needed. After three weeks of uneventful recovery, the erythrocytosis disappeared (PCV: 0.36, haemoglobin: 126 g/L), and the patient remained symptom-free on long term follow-up examinations.

Comments and conclusions

In children who present with polycythaemia a thorough evaluation is needed (120), thus peculiar multiple associations – e.g. Wilms tumor, polycythaemia, ear malformations and ichthyosis, possibly a new syndrome (44) – can also be detected.
Children with unexplained polycythaemia should be investigated for Wilms tumor even in the absence of elevated serum erythropoietin. Out of 10 cases, 7 patients were more than 16 years of age, 8 were boys, and 9 were clinical stage I with favourable histology (71). Unexplained erythrocytosis should sound the alert for further diagnostic studies to evaluate the possibility of occult renal neoplasia (114).

- **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 erythrocytosis.**
- **Children with unexplained polycythaemia should be investigated for Wilms tumor, a potentially curable disorder.**
- **Finally (for educational purposes): after reviewing the pertinent literature we can state that designations such as "erythrocytosis" and "polycythaemia" can be used interchangeably.**

CHAPTER 2 – THERAPEUTIC ISSUES

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 (56)

2.1. Good response to a new therapy in a childhood chronic myeloid leukaemia

Background

Chronic myeloid leukaemia (CML) is a clonal disorder which appears in less than 5 percent of all childhood leukaemias. The majority of paediatric cases are diagnosed after the age of 4 years. It is caracterised by myeloid hyperplasia of the bone marrow, extramedullary haematopoiesis, elevation of the leukocyte count and a specific cytogenetic marker,the Philadelphia (Ph1) chromosome(except in juvenile type of CML). The characteristic genetic abnormality of CML is the reciprocal translocation of the long arms of chromosomes 9 and 22, – t(9:22)(q34:q11) – 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. As a standard treatment busulphan or hydroxyurea and/or interferon-alpha is used with an overall survival of around 5 years. Allogeneic bone marrow transplantation improved the results but it is associated with substantial morbidity and mortality and is limited to patients for whom a suitable donor is available.
The new therapeutic agent STI 571 (formerly known as CGP 57148B), imatinib (as its mesylate salt: imatinib mesilate), is a competitive inhibitor of tyrosine kinase. Clinical trials are ongoing in Hungary and in other countries with promising results (31, 34). There are, however, very few data on experience with imatinib in pediatric practice (52, 62).

Case report

In December 1998, a 12-year-old boy was admitted for evaluation because of excessive splenomegaly.On admission, physical examination revealed a spleen extending into the true pelvis, but no other mentionable alteration. Leukocyte count was very high: $336x10^9$/L, platelets: $524x10^9$/L,haemoglobin:117g/L.The only pathological finding in blood chemistry was the elevated serum lactate dehydrogenase level: 1940 U/L. The bone marrow examination proved chronic myeloid leukaemia in accelerated phase with diminished leukocyte alkaline phosphatase activity. Philadelphia chromosome positivity (Ph+) was found in 48%. RT-PCR detected b3/a2 mRNA type (with the third exon of the M-BCR region of the bcr gene linked to the second abl exon).
Hydroxyurea treatment was started lasting for 6 months. There was a considerable but transient decrease in the size of the spleen, in leukocyte count and lactate dehydrogenase level, but Ph+ 47% and molecular alteration persisted. Treatment was changed for interferon-alpha (3x5 MU / week). Because of an increase in Ph+ (65%), interferon dose was increased to 5 MU / day. Considering the negative result of the interferon resistance test, a trial of higher dose interferon (9 and 5 U / day, respectively, on alternate days), was initiated. Although interferon treatment seemed to be ineffective (regarding the 76% Ph+, i.e.cytogenetic-molecular unresponsiveness) and only a moderate clinical and haematologic improvement, interferon dose was once more increased (9MU daily dose) for five more months. After two and a half years of administration, interferon treatment was discontinued. During this period, an active search for allogeneic bone marrow donor was performed without success.
Three years after diagnosis, in December 2001 imatinib treatment started: 400 mg/day in four oral doses.

From the third week of therapy excellent haematologic and molecular-genetic responses were detected. Ph-negativity persisted for 11 months, bone marrow was regularly examined in 2-4 months

intervals. A slight Ph+positivity, and a hybridization sign corresponding to bcr/abl-fusion detected in 7 percent of cells, a value just exceeding normal background positivity, were found by the end of the first year of imatinib treatment. The only side effect of the therapy was a transient myalgia, but the patients quality of life remained excellent. In the third year of treatment, characterised by stable clinical condition, haematologic remission and Ph-negativity and a low level (9%) bcr/abl positivity were found on a single occasion. It could probably be attributed to a compliance problem: oral medication four times a day for an evidently healthy adolescent. Therefore therapy was changed for a single daily dose of 400 mg imatinib. In 2005, at the end of the fourth year of targeted therapy, and seven years after the diagnosis of his primarily refractory, practically incurable illness, the patient lives a normal, healthy life in complete clinical, haematologic and molecular-cytogenetic remission.

Discussion, conclusions

Targeted therapy (105) or molecularly targeted therapy (136) uses an array of new agents produced as a consequence of deeper insights into the molecular pathogenesis of cancer (23). These new agents are more selective in hitting their targets,and so their use will be more narrowly defined than with classical cytotoxic drugs. Two main groups can be distinguised: small molecule inhibitors and monoclonal antibodies. The representatives of these inhibitors are the proteasome inhibitor bortezomib (Velcade), tyrosin kinase (TK) inhibitor imatinib (Gleevec) inhibiting both bcr/abl TK and c-kit receptor TK, and also platelet derived growth factor receptor TK. Imatinib is used in chronic myeloid leukaemia, in gastrointestinal stromal tumors and in a number of other malignancies. Monoclonal antibodies target growth factors, their receptors, e.g.epidermal growth factor receptor „inhibitor" cetuximab (Erbitux),vascular endothelial growth factor „inhibitor" bevacizumab (Avastin) acting in cancers expressing the specific antigen (colorectal cancer, metastatic processes). An example of specific monoclonal antibodies (anti CD20, rituximab) will be discussed later; a bevy of therapeutic monoclonal antibodies and signal transduction inhibitors also belong to the group of „new agents".
The question whether imatinib or allogenic haematopoetic cell transplant should be the front-line therapy, in paediatric CML, remains unanswered . Both imatinib and reduced intensity conditioning (RIC) stem cell transplantation are promising tools offering potential decrease in therapy associated morbidity. The latter approach has yet to be verified in phase III studies, which should be planned carefully in order to avoid sub-optimal outcomes (98).
Imatinib treatment of paediatric CML can carry the risk of failure, e.g. blastic transformation:the patient received ALL-type chemotherapy followed by allogeneic stem cell transplantation, successfully. Authors concluded that CML patients who respond (slowly) to imatinib may still be candidates for allogeneic stem cell transplantation, even when a major cytogenic response is obtained (85).
In an other case of childhood Philadelphia chromosome-positive (Ph+) acute lyphoblastic leukaemia – a possible indication for imatinib therapy – a progressive resistance to imatinib developed, and it was correlated with the in vitro resistance assay and the appearence of a missence mutation of T to C (Y253H) of the ABL gene, which may play an aetiological role in the loss off drug sensitivity (57). An other mechanism of resistance might be the induction of the ABCG2 (BCRP) and ABCB1 (MDR1) drug transport proteins. These ABC transporters are normally expressed in the gastrointestinal tract and upregulation of these pumps – proven in vitro by chronic imatinib exposure of CaCo2 (human colon carcinoma) cells – may reduce oral bioavailability of imatinib, leading to drug resistance in cancer patients that are chronically treated with imatinib (14).

- **Based on the above facts, a cautious and reserved attitude seems to be appropriate during chronic imatinib treatment.**
- **Our case demonstrates that imatinib could be a safe and effective strategy in the treatment of adult type CML in children.**
- **When deciding in favour of imatinib treatment (18 months before the paediatric approval by FDA) we had to face very hard challenges e.g. professional, ethical, and financial issues (not discussed in detail).**

- **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 pediatric leukaemia by US Food and Drug Administration in 2003 (88).**

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Malignant (strictly spoken: non-Hodgkin) lymphomas derive from cells of the immune system as a consequence of alterations that influence their proliferation, differentiation and ability to undergo apoptosis. They can be classified by histopathological evaluation including immunophenotyping, and can be divided into B- and T-cell types with certain difference in tissue or organ involvement, clinical course and prognosis. The latter was extremely poor before the 1960s, with a 5-year survival of 5 to 33 percent. As a result of progress in treatment modalities, use of protocols developed by childhood cancer study groups (e.g. BFM, NCI, POG), the prognosis of malignant lymphomas improved greatly in the last two decades, approaching 90 percent cure when treated optimally: applying combined modalities, including immunotherapy, and autologous stem cell rescue as well, in certain entities, in refractory or relapsing cases.

2.2. Refractory B-cell lymphoma successfully treated with ritaximab and autologous stem cell transplant.

Background

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. In this context, various modalities of immunotherapy including monoclonal antibodies, have been used with promising results (25). Rituximab is a chimeric mouse/human anti-CD20 antibody, acting on CD20 antigen expressed in different B-cell malignant lymphomas, e.g. in all cases of mature B-cell (Burkitt) lymphoma, the most frequent B-cell lymphoma in children , and in diffuse large B-cell lymphoma.
Rituximab as a single agent was systematically investigated in 131 cases of different B-cell malignancies incurable with standard therapy, and a moderate or limited activity was found; its use in combination with cytotoxic therapy is warranted (40). Several, mostly randomised, studies proved the efficiency of this type of combination therapy in non-Hodgkin malignant lymphomas, as shown by increased complete response rate, event-free and overall survival, by decreased failure and death rate without an increase of toxicity (21, 28). Other combinations – interferon alpha 2a and GCSF, and interleukin-2, respectively – also resulted in increased efficacy without compromising tolerability (41, 60). Rituximab has also been used in connection with high dose chemotherapy and autologous stem cell transplant with encouraging remission rates (37).

Case report

The 3-year-old boy was admitted with a rather short history of abdominal pain and vomiting for two days. On physical examination, moderate splenomegaly and extreme enlargement of both kidneys were found. CT examination supported and corresponded to the physical findings, and detected enlarged abdominal lymph nodes, too. Clinical laboratory investigations revealed no mentionable changes except for elevated serum LDH activity. A percutaneous ultrasound-guided needle biopsy was performed: histologically non-Hodgkin lymphoma of diffuse large B-cell immunoblastic type was diagnosed. The tissue sample showed a very intensive raction with anti-CD20 immune serum.
Treatment was started according to NHL BFM 95 protocol. After the first block of therapy a conspicuous regression could be established. The size of the liver and the spleen increased and serum LDH activity rose again after the third block, but symptoms and signs improved somewhat after the next cytostatic block. This fluctuating character of the disease persisted, and by the end of the protocol, definite signs of progression were found.

Because of the apparently refractory disease, rituximab treatment was initiated eight months after diagnosis. The patient was given rituximab, four weekly doses of 300 mg in intravenous infusion – without any signs of toxicity – followed by an autologous stem cell transplant, nine months after diagnosis. Response was satisfactory: signs and symptoms of lymphoma showed a slow but definite regression. Rituximab treatment was then repeated in a similar fashion, resulting in complete recovery. Twenty months after diagnosis, a left-sided pneumonia accompanied by moderate hepatosplenomegaly raised the suspicion of a relapse, but the process caused by pneumococci resolved completely after antibiotic treatment. All the clinical, radiological and laboratory signs disappeared and turned out to be normal, respectively. The child is disease-free on follow up three and a half years after diagnosis.

Remarks and conclusions

Recent publications also support the efficacy of ritaximab and combination treatment (cytotoxic chemotherapy, autologous stem cell transplantation) for non-Hodgkin B-cell lymphoma (29, 48, 51, 110).
Introduction of monoclonal antibodies in combination with chemotherapy may provide a method for improving outcome in poor risk groups and reducing sequelae by allowing reduction in chemotherapy in good risk patients (97).
Rituximab appears safe and modestly effective in a variety of immune-mediated haematologic diseases, e.g. autoimmune haemolytic anaemia, chronic immune thrombocytopenia (81).

- **Continuous and complete remission can be achieved even in apparently refractory childhood B-cell lymphoma applying a relative aggressive chemotherapy protocol followed by "sandwitch" ritaximab therapy together with autologous stem cell transplantation.**
- **Combined treatment can lead to complete recovery and to a possible cure rate exceeding even 90 per cent.**
- **Our case was the first childhood one treated efficiently this way in Hunagary.**

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The efficacy of cytotoxic chemotherapy in children with cancer is highly dependent on the tolerability of the regimes, and of their constituents, i.e. side-effects, toxicity and late effects of the respective drug and of the treatment modalities. 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 (lymphocytes, granulocytes), leading to potentially life-threatening infections.
Toxic lesion of vital organs may manifest as a spectrum of functional disturbances, ranging from an acute emergency state to long term consequences (as mild, moderate or severe even lethal complications and transitory or permanent, manifold late-effects) nfluencing the quality of life of the survivors.
Recognition and management of the untoward effects of the therapy, their possible prevention and/or efficacious treatment – including the development and use of (previously presented) efficient and more tolerable, adjusted, targeted approaches – may lead to the change, even to disappearence of past and present problems connected with infectious or toxic lesions in children treated for cancer.

Children on cytostatic, immunosuppresive 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.

2.3 Immunization against varicella of children receiving cytostatic, immunosuppressive treatment. A historical perspective

Background

Varicella has been considered as a mild illness by both doctors and patients. Investigations in the past two decades revealed, however, that chickenpox can be dangerous and even lethal in immunosuppressed patients and in children in poor condition due to a malignant disease (13, 87)
Passive immunization by zoster immunoglobulin was a great step forward. This possibility was investigated in Hungary by Nyerges et al (90). In 54 children with negative varicella history and negative anti-varicella antibodies receiving immunosuppressive therapy, we performed continuous varicella prevention. During a period of one year, the children were administered every 6 weeks i.m. Varicellon injections, an immunoglobulin prepared from the serum of healthy donors containing high titre anti-varicella antibodies. From the 54 cases, varicella exposition was known in 18 patients. Mild varicella developed in 12 children. As a conclusion: this form of passive immunization was effective in preventing high-risk manifestation of the disease, however secures only partial prevention of varicella. Passive immunization until remission occurs, and active immunization during remission may be considered as more efficacious. (V). Therefore, regarding the moderate efficiency of the passive procedure, it was important to investigate the possibility of active immunization, especially in high-risk cases (IV).

Investigations and Results

During five hospital epidemics, Takahashi OKA live-attenuated varicella vaccine was administered to 33 patients – having their susceptibility confirmed by serological examinations – either subcutaneously (14 pts, 0.5 ml) or intracutaneously (19 pts, 0.1 ml) after randomisation, without mentionable local or general untoward reactions.
One month later seroconversion was detected in a somewhat greater proportion in the subcutaneous group: 12/13 versus 10/13. Subcutaneously vaccinated children contracted mild varicella in 2/14 cases, and in 3/19 intracutaneously vaccinated patients, respectively, between days 10 to 17 after vaccination.
The limited number of cases involved in our investigation does not allow definite conclusions, nevertheless, a great advantage of the vaccine is that – although it should be administered the soonest possible – it is effective even after exposure. The OKA vaccine of Takahashi has been demonstrated to be safe in patients with leukaemia and other malignant diseases. Still, as immunosuppressive cytostatics decrease the degree of immunization, it has been generally accepted that such patients should be tested serologically for immunity and in case of susceptibility they should be given varicella-zoster immunoglobulin (VZIG) treatment. 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-BFM 95) focuses on acyclovir for both prevention and treatment.

Discussion, recent developments and conclusions

Impaired immunity, T-cell deficiency (e.g. resulting from chemotherapy, more frequently in ALL as compared with solid tumors) historically proved to lead to severe visceral spread – pneumonitis, hepatitis, encephalitis – in about 30 percent of varicella cases, with a mortality rate of 7 to 20 % (33, 35, 89). Post exposure prophylaxis with VZIG and/or intravenous antiviral therapy clearly improved the outcome in these patients, however, also Acyclovir treated children – 3/69 more than 4 % - died of varicella: varicella mortality could be favourably modified through on active immunization of immunocompetent children (38).

Over the past 10 years, varicella vaccine has been given to millions of children in the United States, usually at ages between 12 and 18 months, resulting in marked decrease of reported cases of varicella, furthermore, a 75% decrease in varicella-related hospitalisation and a similar decrease in the number of deaths caused by complications of chickenpox. An unanticipated result, the growing number of outbreaks of varicella among immunized children ("breakthrough varicella") can be explained by the lessened immune reponse at 12 months of age, and genetic variants among circulating VZV strains (42). The most compelling rationale for introducing universal vaccination, the predicted benefits for healthy children, are being realized (49). Varicella deaths (around 100 per year in the prevaccination era) decreased markedly – around 40 per year – for all age groups (16, 17, 89).

As varicella can be fatal and some deaths among healthy children continue to occur despite the availability of a safe and effective vaccine (124, 125), by June 2004 most (44) States in the USA had implemented elementary school entry requirements for varicella vaccination, but middle or high school entry requirements are also needed (17).

It is not quite clear from earlier investigations – which state that naturally acquired prior immunity to varicella is compromised during and/or after antineoplastic therapy – whether immunologic protection acquired by prior vaccination against varicella will be a persistent, efficient one (101). Regarding the immunocompromised individuals: some of the current recommendations are based on few and small studies with short follow-up. Therefore, the varicella vaccine should be given only with complete knowledge of their clinical and immunological conditions, and after considering risks of natural infection and vaccination (109).

As early as in 1996, 575 leukaemic children in remission were vaccinated, chemotherapy stopped one week before and one week after immunization. Of 123 exposed children, 17 (14%) developed mild varicella; the vaccination protected completely against severe varicella (73). It is of note that recent steroid therapy increases the severity of varicella infection in children with ALL; therefore, with the probable exception of induction therapy, patients who are exposed to varicella should have steroid treatment delayed until after the VZV incubation period (46).

In an other recent study seronegative cancer children received two doses of live-attenuated varicella vaccine in a span of three months: 13 ALL children in the maintenance phase of chemotherapy and three solid tumor cases around 3-6 months after treatment discontinuation. VZV specific cellular and humoral immune responses improved substantially with a seroconversion rate of 94 %. One subject developed probably vaccine-related chickenpox (77).

It is worth of consideration, however, that both general practitioners consultation rates for chickenpox and the incidence of chickenpox are declining even in the absence of universal childhood immunization (80).

- **In principle: varicella vaccine should not be administered to patients who have a cellular immunodeficiency, but persons with impaired humoral immunity may be vaccinated (16).**

Vaccination recommendations for immunocompromised patients by NACI (89):

"Isolated" immunodeficiency (immunoglobulin, neutrophil, complement) or asplenia: vaccinate as for healthy

ALL in remission, asymptomatic HIV infection (CD4 > 25%), patients awaiting kidney or liver transplant, patients more than 2 years after bone marrow transplant, or at least 3 months after being cured of malignant diseases: may vaccinate*

Patients receiving immunoglobulin or blood: vaccination should delay

In solid tumor and other instances of immnosuppresive therapy: vaccination is not recommended

T-cell deficiency (SCID, AIDS): vaccination contraindicated **

(*, ** explanation: next page)

* VZIG is considered after exposure if VZ antibodies are not present
(VZIG is not needed if antibodies are presentsent)
** VZIG (compulsory) after exposure

- **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 concerns regarding efficatious prevention and treatment .

2.4 Successful treatment of aspergillus infection in a child with (secondary) malignancy

Background

Major factors predisposing to fungal infection (neutropenia – its duration and severity – and intensive antibiotic treatment) together with airborne and/or direct contamination, can lead to severe respiratory tract infection caused by Aspergillus species.After Candida species, Aspergillus species constitute the second most common fungal pathogens in the immunocompromised host. Upper airway colonisation precedes most cases of invasive infection(1, 137).

Case report

The 13-year old girl was hospitalised because of abdominal complaints.A tumorous mass from the ileocecal region,together with adjecent lymph nodes was removed surgically. The child was previously, efficiently treated for ALL at 2 years of age. On regular follow-up visits a continuous complete remission was ascertained, and cure could be declared. Thus, the latter process has been regarded as a second tumor. Histologically a monocytic/histiocytic sarcoma was diagnosed with signs of plasmocytoid differenciation. Treatment was instituted according to AML-BFM 98 protocol,and continued for five months when profound neutropenia (ANC<100/microliter) ensued, and respiratory tract symptoms (dyspnoe, coughing) appeared with moderate fever. Chest X-ray film showed a solid, tumor-like mass and infiltration, respectively, in the right lung. A malignant process (metastasis ?) was suspected and bronchoscopy performed. The specimen obtained proved to be a necrotic mass with numerous fungal septated hyphae histologically. The fungi were identified morphologically and with culture as of the Aspergillus species. The condition of the patient deteriorated, therefore the cytostatic treatment was interrupted and a 10 days course of amphotericine B treatment was administered.

The clinical course was that of a relatively rapid improvement,and the radiologically proven lesion disappeared.
As the otherwise lethal complication of aggressive cytostatic treatment responded excellently to the immediate institution of specific antifungal therapy, chemotherapy protocol for the underlying malignancy could be continued. Three months later, the malignant process turned out to be refractory, change for an other protocol – NHL-BFM 95 – and treatment with local hyperthermia pulses, as well, were applied with partial success to slow down progression. The patient died 18 months after the diagnosis of histocytic sarcoma as the second malignancy.

Conclusions and comments

- **During the agressive cytostatic treatment, we have to recon also with severe fungal infections, too.**
- **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.**
- **The fungal process described might correspond to an initial stage of invasive aspergillosis, which – according to our observation – can respond well to standard antifungal chemotherapy.**

The angiotropism (i.e. propensity to invade blood vessels) of Aspergillus species can lead to necrosis and systemic dissemination .Variconazole, a second generation triazole has been recently shown to lead to better response and beeing less toxic than amphotericin B, and can be applied for combination antifungal therapy (1). As to the current approaches: antifungal therapies include amphotericin B deoxycholate, lipid formulation of amphotericin B, the triazoles (fluconasole, itraconazole and variconazole) and the echinocandins (caspofungin) and investigational agents micafungin, amygdalafungin.

Variconazole is recently regarded as the initial choice of treatment in most patients with invasive aspergillosis. If patients are intolerant or refractory to therapy, effective alternatives include a lipid formulation of amphotericin B or an echinocandin (93)

Reports are very limited in the pediatric population, nevertheless, a child with relapsed ALL who developed aspergillosis and subsequent multiorgan dissemination during therapeutic induction, was treated successfully with caspofungin acetate (9).

Amphotericin B resistance both in vitro and in vivo is more freqent in invasive aspergillosis caused by a peculiar strain, Aspergillus terreus (occuring almost in the same percentage – 48% – of cases as all the other „non-A-terreus" species in a single institution), associated with a high rate of dissemination and poor outcome (74).

There is currently no evidence of a definite and universal antifungal profilaxis strategy effective againts asprgillosis in leukaemic patients accounting for a majority of aspergillosis cases: clinical trials on prophylaxis should also be designed for patients with leukaemia (45).

*

Besides therapeutic trials, the introduction of novel modalities, and efforts to realize and manage complications (e.g. prevention and treatment of infections) affecting immunocompromised patiens, therapy-related problems in the management of childhood cancer involve also the recognition, examination and proper handling of early, acute and chronic, late toxicities and side effects.

Our studies ranged over a relatively narrow field of these problems.

2.5 Toxicity of high dose methotrexate treatment

Objective

Toxicity was studied in frame of our clinical survey of high dose methotrexate (HD-MTX) treatment (VI). When HD-MTX is administered, the single dose (g/m^2) will be two orders of magnitude higher then the "classic", oral dose (10 $mg/m^2/day$), so we have to recon with increased toxicity.

Patients, methods, results

To assess the toxicity of the treatment, data concerning 103 treatments in 26 paediatric patients were analyzed based on the modified WHO toxicity score (138). In this procedure, each component (symptom, sign) will be assigned 0 point if absent, and 1 to 4 points if present, according to the grade of severity. A total of points divided by the number of treatments will numerically characterize the toxicity of the given – in our case: HD-MTX – treatment, as regards individual symptoms, organ manifestations. The simplified table below shows this "mean symptom weight" (i.e. "symptom-severity") and the number of patients having the given symptom.

renal lesion	0.87	20 patients (of 26)
vomiting	0.82	21 pts
leukopenia	0.71	20 pts
hepatic lesion	0.3	16 pts
mucosal lesion	0.3	9 pts
thrombocytopenia	0.16	6 pts
skin lesion	0.04	1 pt

Relying on these findings, in a significant proportion of patients renal or hepatic lesion, vomiting, leukopenia occurred, but all the toxicity symptoms and signs were mild, on overage below 1 (grade one).
Besides these 7 types of relatively mild side effects, remarkably, no diarrhoea or alopecia attributable to HD-MTX, no signs of infection or other organ lesions (nervous system, cardio-, oto-, pulmonary toxicity) occurred. All these facts warrant the feasibility of preventive measures, the importance of close observation and supportive care.
"Toxicity index" of individual patients was also studied, derived as a quotient of the total symptom-score (sum of points) divided by the number of HD-MTX treatments.
This value – based on 12 "kinds" of expectable HD-MTX toxicity – can mount up to 48, and up to 28 in our cases, based on 7 "kinds" of toxicity "symptoms" observed by us.
"Toxicity index" was evaluated in 23 patients during 86 treatment cycles, as shown below in three groups of patiens according to the single dose of methotrexate.

			„toxicity index"	(range)
2 g/m^2	5 patients	20 treatments	2.80	(1.0-4.5)
5 g/m^2	16 patients	52 treatments	4.26	(0.5-9.0)
12 g/m^2	2 patients	14 treatments	2.50	(0.5-4.5)

No close correlation with the dose was found and, like as before, both the mean and upper values of the range of "toxicity index" were low in each group. (Because of the limited number of cases impact of diagnosis, duration of the infusion, and variations in folinate-rescue were not analyzed.)

Conclusion

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

2.6 Late effects on renal function in childhood cancer survivors

Background

As the proportion and absolute number of childhood cancer survivors increase, the importance of the late effects of the disease and its treatment, influencing health-related quality of life also increases. Among the manifold (e.g. cardiovascular, endocrine, growth, hepatic, obesity, osteoporosis, neurocognitive, psychosocial, pulmonary, and second neoplasm) sequelae, as an example, renal manifestations, i.e. 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.

Study objectives, methods and results

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. Patients were enrolled in three groups: leukaemia – lymphoma (60 cases), Wilms tumour (22cases), and other solid tumours (33 cases). All patients were treated according to standard protocols applied by the Hungarian Paediatric Oncology Group. 86 children without any renal or urinary tract disease were studied as controls.
Besides routine physical, laboratory and ultrasound examinations, serum cystatin C, serum and urine creatinine concentrations were measured, and the glomerular filtration rate calculated. Urinary N-acetyl-beta-D-glucosaminidase (NAG) enzyme activity (normalized for urinary creatinine concentration as NAG indices, NAGi) and microalbuminuria, as well as insertion/deletion polymorphism of angiotensin converting enzyme (ACE) was also determined.
In contrast to leukaemia-lymphoma and (other) solid tumour survivors, significantly elevated cystatin C, mildly elevated creatinin concentrations in serum, and significantly lower glomerular filtration rate values were found in Wilms tumour patients (Fig.10) Each Wilms tumour survivor had advanced disease requiring complex tratment.

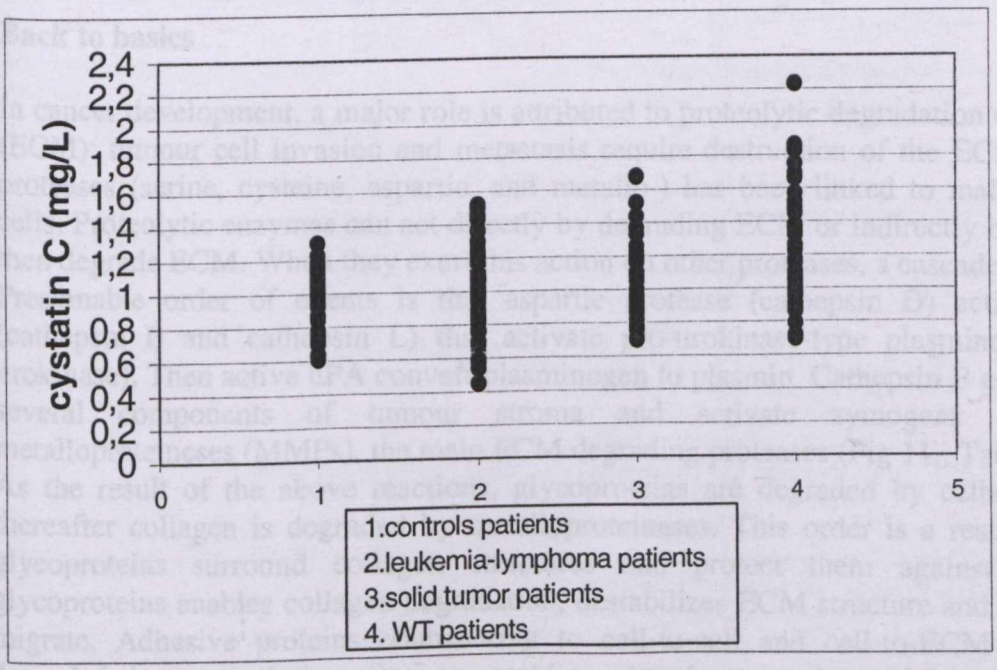

Fig.10. Distribution of cystatin C concentration of controls, leukaemia-lymphoma, solid tumour, and Wilms tumour (WT) survivors representing GFR glomerular function parameters. Closed circles represent actual patient and control values. Shaded areas indicate the reference range. Lines represent mean values ±1 SD. Open square indicates cystatin C concentrations of HR WT patients. There was a statistically significant difference ($P<0.05$) in cystatin C concentrations of WT patients and controls.

Out of 30 patients with gross proteinuria this feature disappeared in 20 cases spontaneously, so proteinuria persisted in ten patients: 4 (out of 60) leukaemia-lymphoma, 4 (out of 22) Wilms tumour and 2 (out of 33 other) solid tumour cases, about three times more frequent in Wilms tumour, than in other groups). Degree of proteinuria correlated significantly with microalbuminuria but not with otherwise elevated NAG excretion. Persisting proteinuria progressed in three cases, they were put on ACE – inhibitor therapy with almost complete success.
ACE genotypes and allele frequencies of patients with proteinuria did not differ significantly from controls.
Pathologically elevated urinary NAGi and miroalbuminuria values, respectively – indicating impaired proximal tubular function – were noted in 38 respectively 16 % of leukaemia-lymphoma cases, in 20 respectively 5 % of Wilms tumour, and in 54 respectively. 25 % of solid tumour survivors: the latter was statistically significantly different from controls.

Conclusions

- **We found less frequent and less severe late nephrotoxic side effects than others; nevertheless mean follow-up time in those studies was shorter (1-48 months) than ours: 86 months (6, 53, 63).**
- **Mild to moderate subclinical glomerular and tubular damage could be identified in many childhood cancer survivors; most patients, however, experienced some spontaneous recovery.**
- **Due to the routinely applied supportive measures during chemotherapy, development of significant renal abnormalities is an exception rather than a rule.**
- **Patients at risk should be identified and subjected to life-long check-up programmess to detect and manage kidney-associated late morbidity and thus preserve the quality of life**

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CHAPTER 3 – BIOCHEMICAL TOPICS

Back to basics

In cancer development, a major role is attributed to proteolytic degradation of the extracellular matrix (ECM): tumour cell invasion and metastasis require destruction of the ECM. Every known class of proteases (serine, cysteine, aspartic, and metallo-) has been linked to malignant invasion of tumor cells. Proteolytic enzymes can act directly by degrading ECM or indirectly by activating others which then degrade ECM. When they exert this action on other proteases, a cascade will lead to proteolysis.
Presumable order of events is that aspartic protease (cathepsin D) activates cysteine proteases (cathepsin B and cathepsin L) that activate pro-urokinase-type plasminogen activator (pro-uPA, urokinase). Then active uPA convert plasminogen to plasmin. Cathepsin B as well as plasmin degrade several components of tumour stroma and activate zymogens (precursors) of matrix metalloproteineses (MMPs), the main ECM degrading proteases (Fig 11., Table 2.).
As the result of the above reactions, glycoproteins are degraded by cathepsins and plasmin first, thereafter collagen is degraded by metalloproteinases. This order is a result of ECM composition: glycoproteins surround collagen structures and protect them against proteolysis. Removing glycoproteins enables collagen degradation, destabilizes ECM structure and allows neoplastic cells to migrate. Adhesive proteins contributing to cell-to-cell and cell-to-ECM adherence will also be degraded by proteolytic enzymes enabling detachment of neoplastic cells from the tumour. Degradation of the ECM proteins and the basement membrane (BM) contribute to cancer development (117).

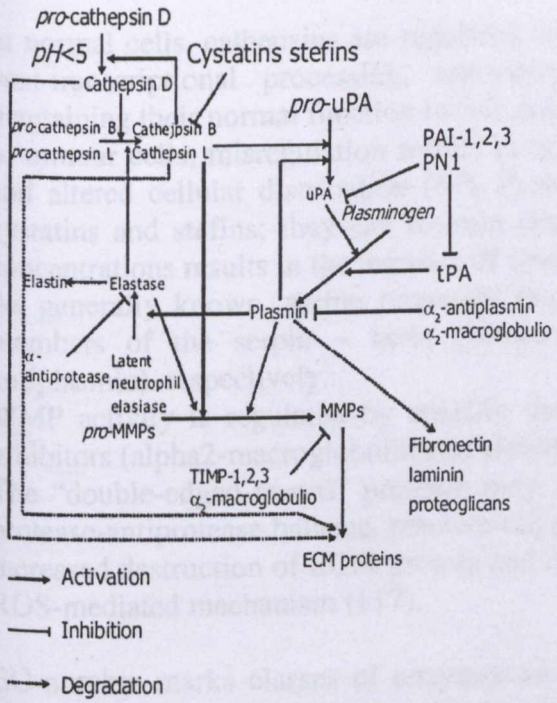

Fig 11. Regulation of ECM proteolysis

Protease family	Protease	Protease function	Protease inhibitors
Aspartyl protease	Cathepsin D	Degradation of ECM components Conversion of cysteine procathepsins into cathepsins	
Cysteine proteases	Cathepsins B, L, H, K	Degradation of ECM components Conversion of pro-MMPs into MMPs	Cystatins, stefins, kininogen
Serine proteases	Plasmin	Degradation of ECM components Activation of uPA Conversion of inactive elastase into elastase	α_2-antiplasmin α_2-macroglobulin
	Urokinase-type plasminogen activator (uPA)	Conversion of plasminogen into plasmin	PaI-1, 2, 3
	Tissue-type plasminogen activator (tPA)	Conversion of plasminoge into plasmin	
Neutrophil serine proteases	Elastase	Degradation of ECM components	α_2-antiplasmin
	Cathepsin G		α_2-macroglobulin secretory leukoprotease inhibitor
Matrix metalloproteinases		Degradation of collagens and other ECM proteins	TIMP-1, 2, 2, 4
		Activation another pro-MMPs into MMPs Degradation of collagens: I, II, III, VII, X and gelatins	α_2-macroglobulin
	Collagenases [MMP-1, 8, 13]	Degradation of proteoglycans, laminin, gelatins, collagens III, IV, V, IX, fibronectin, entactin, SPARC, collagenases-1	
	Stromelysins [MMP-3, 10]	Degradation of gelatins, collagens: I, IV, V, VII, X, fibronectin, elastin, procollagenase-3	
	Gelatinases [MMP-2, 9]	Degradation of collagen I, II, III, gelatins, aggrecan, fibronectin, laminin, vitronectin, MMP-2, 13, tenascin, nidogen	
	Membrane-type [MMP-14, 14, 16, 17, 24, 25]	Degradation of proteoglycans, laminin, fibronectin, gelatins, collagens IV, elastin, entactin, tenascin, α_1-antiproteinase, amelogenin	
	Others [MMP-7, 11, 12, 19, 20, 23]		

Table 2. Proteases participating in degradation of ECM com

In normal cells, cathepsins are regulated at every level of their biosynthesis (including transcription, post-transcriptional processing, translation, post-translational processing and trafficking) thus mantaining their normal function in cell metabolism.
In tumour cells, misregulation results in increased mRNA and protein expression, increased activity and altered cellular distribution (67). Proteolytic activities of cysteine proteinases are inhibited by cystatins and stefins; they can restrain tumor cell invasion and metastasis, and a decrease of their concentrations results in the increase of cysteine cathepsin activity (10).
As generally known, serine proteases (e.g. plasminogen activators and plasmin) are inhibited by members of the serpin – serin protease inhibitor – super-family (PAI-1, PAI-2 and alpha2-antiplasmin), respectively.
MMP activity is regulated by specific tissue MMP inhibitors (TIMPs), and non-specific protease inhibitors (alpha2-macroglobulin and alpha1-antiprotease).
The "double-edged sword" problem may also be mentioned here: among other factors influencing protease-antiprotease balance, reactive oxygen species (ROS) can inactivate antiproteases resulting in increased destruction of ECM protein and in metastasis; while malignant cells can be killed through a ROS-mediated mechanism (117).

EC number marks classes of enzymes and subclasses defined according to the reaction catalyzed. Hydrolases (EC3) acting on peptide bonds: peptidases (EC 3.4) comprise – according to the active center – serine endopeptideses (EC 3.4.21), cysteine endopeptideses (EC 3.4.22), aspartic endopeptideses (EC 3.4.23), and metallo-endopeptideses (EC 3.4.24). Representatives of the previous three group are also called cathepsins, to the fourth group belong matrix metalloproteinases, MMPs (11). Enzymes studied by us are cysteine endopeptidases, namely cathepsin B (EC 3.4.22.1), cathepsin H (EC 3.4.22.16), cathepsin L (EC 3.4.22.15) and matrix metalloproteinase 7 – synonym: matrilysin – (EC 3.4.24.23). Designations such as peptidases, proteases, proteinases, proteolytic enzymes are considered as synonyms.

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3.1 Activity of cathepsin B, H, L and MMP 7 in the serum of children with ALL and tumors

Background

Earlier studies (78, 110, 119, 131, 135) detected increased activities of cysteine proteineses (cathepsins) and metalloproteinases in malignant tissue samples as compared with non malignant ones. A correlation with metastatic poteintial was suspected (78, 119). No data concerning serum enzyme activities in paediatric cancer (ALL and solid tumours) were found in the pertinent literature before our investigations.

Patients, methods and results

We studied cathepsin B, H, L and matrix metalloproteinase 7 (MMP 7) enzyme activities in the serum of 30 childhood cancer patients: ALL: 22, solid tumour: 8 cases. The control group comprised 13 healthy children. Proteinase activities of serum were measured using different amino acid – 7-amino-4methyl-coumarin (AMC) substrates. Fluorescence by liberated AMC was measured spectrofluorometrically. Details of the biochemical methods can be found in the original publication (VIII).

Enzyme activities (mU/ml, meau ±SD)

Group	Cathepsin B	Cathepsin H	Cathepsin L	MMP 7
ALL n=22	11.8±7.3	170.8±51.0	1.69±0.8	5.9±2.1
P value	NS	<0.001	NS	í0.005
Tumours n=8	20.6±8.2	134.3±56.3	1.0±0.6	9.9±10.4
P value	<0.05	<0.05	NS	NS
Controls n=13	10.3+3.7	89.6±33.6	1.3±0.8	8.4±2.6

Conclusions and remarks

- **A significant increase in lysosomal cysteine proteinase, i.e.cathepsin H, was demonstrated in serum of ALL patients, either in the initial intensive treatment period or in remission or during maintenance treatment.**
- **In the solid tumours' group, elevated cathepsin B and H activities were found in accordance with earlier observations (100, 119).**
- **Our data do not support the role of metalloproteinase-7 in malignant conversion (similarly to that in case of cathepsin L), as no elevated enzyme activity was detected in sera of children suffering from ALL and tumours.**
- **Enzyme activities expressed numerically were widely scattered in various tumour bearing children, the ALL group, however, was rather homogenous.**
- **Since there were no data available on these serum enzyme activities before the treatment, the role of chemotherapy in the elevated cathepsin H activity can not be excluded.**
- **From a comparison of the elevated serum cathepsin enzyme activities with the clinical data on the children (most of them (25/30) under aggressive cytostatic treatment),we can assume a role of cell damage in this phenomenon.**

In physiological conditions, equilibrium between proteases and their inhibitors exists in the organism. Cancer development is followed by a temporary decrease of proteolytic enzymes in the cancer cells with simultaneous increase in activity of proteases and decrease in activity of inhibitors in blood serum (117): providing a rationale for investigations of serum of patients.
Cysteine proteinases (cathepsins) examined in tissue samples and body fluid of different carcinoma bearing patients showed a high prognostic impact for the survival (70). Further recent investigations underline the importance of serum protease activity in cancer patients; serum cathepsin D and/or PSA levels together with biopsy, proved to be a useful predictor of the extent of prostate cancer (84); evaluation of the concentration of MMP 2 and its tissue inhibitor in blood serum of patients seemed to be useful for differentiation between benign and malignant thyroid tumours (92); cathepsin B in the blood serum of superficial bladder cancer was significantly enhanced and can be applied for diagnosis (123).
Besides peripheral blood, the tumour tissue is a reliable source of protease detection. Expression of MMP 7 (mainly produced by the cancer cell itself) examined immuno-histochemically in tumour samples, was significantly stronger in high-grade than in low-grade tumours (83).

*

As regards the ever growing amount of numerous (or rather innumerable) publications

cited in PubMed –	from **1950 to 2005**	**in 2005**	
on proteases:	281339	**8739**	
on proteases AND cancer:	35861	**2064**	
on proteases AND cancer AND child*:	1103	**44**	items

on average about 5000, 650 and 20 publications per year.

Therefore one can recon with the growing importance of the topic from diagnostic, prognostic and – hopefully – from therapeutic point of view, also in connection with childhood cancer management.

3.2. Methotrexate inhibits the glyoxalase system in vivo in children with acute lymphoid leukaemia

Background

Methotrexate (MTX) is used in the treatment of different types of cancer. It is a folic acid analogue blocking the enzyme dihydrofolate reductase, thus the conversion of dihydrofolate to tetrahydrofolate, thereby inhibiting the synthesis of DNA. It also inhibits thymidylate synthase, preventing the production of deoxy-thymidylate. The drug also affects normal cells, leading to side-effects such as gastrointestinal toxicity. In a previous pilot study, we tested the plasma D-lactate level as a possible indicator of injuries to the intestinal mucosa - see also (122) – and that study included some patients undergoing high-dose MTX treatment.
D-lactate is a product of the alpha-oxoaldehyde metabolism. The alpha-oxoaldehydes glyoxal and methylglyoxal are formed by lipid peroxidation, glycation and degradation of glycolytic intermediates (127). They bind readily to nucleic acids and proteins and form stable adducts inducing mutagenesis, apoptosis, protein degradation and the formation of advanced glycation end-products. Several enzymes are capable of metabolizing alpha-oxoaldehydes, but the highly active glyoxalase system is considered the main route of methylglyoxal detoxification. The system consists of two enzymes, glyoxalase I (a carbon-sulfur lyse: lactoyl-glutathione lyase, EC 4.4.1.5) and glyoxalase II (a thioester hydrolase: hydroxyacylglutathione hydrolase, EC 3.1.2.6), and a catalytic amount of glutathione. The metabolic flux through this pathway results in the production of D-lactate (Fig.12).

The sources of plasma D-lactate are the endogenous alpha-oxoaldehyde metabolism, the enteric microorganisms and fermented foodstuffs.

In the pilot study (mentioned earlier), in connection with MTX treatment we found a marked fall in plasma D-lactate level (data not shown), 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 decrease in 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 (26).
These data prompted a systematic study of the influence of high-dose MTX therapy on the level of plasma D-lactate in patients with acute lymphoid leukaemia (ALL). The effects of MTX, folic acid and folinic acid (leucovorin) on the activity of glyoxalase I were tested *in vitro* using human erythrocyte lysates or yeast enzyme.

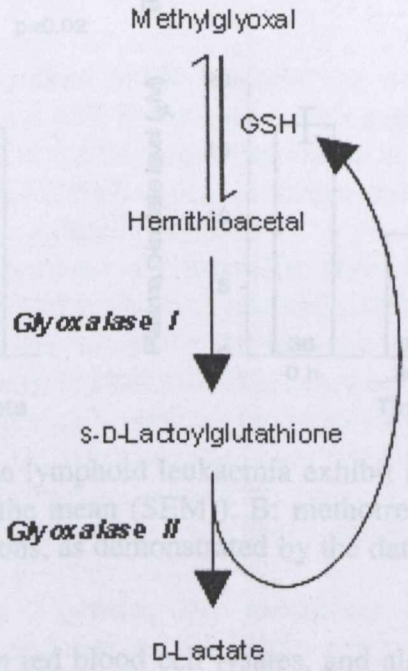

Fig 12. The metabolic pathway of the glyoxalase system. The non-enzymatic reaction of methylglyoxal with reduced glutathione (GSH) produces the hemithioacetal. Glyoxalase I catalyzes the intramolecular redox reaction of the hemithioacetal forming the thioester, S-D-lactoylglutathione. In the last step, glyoxalase II hydrolyzes the ester to D-lactate and GSH

Patients, methods and results

Ten children with newly-diagnosed ALL were treated according to the ALL-BFM 95 protocol. In the phase of consolidation therapy they received high-dose MTX infusions (5 mg/m^2/24h every 14 days, four times), followed by folinic acid rescue (15 mg/m^2 intravenously at 42, 48 and 54 h after the start of the MTX infusion). In each patient 4 consecutive MTX cycles were followed. Blood was collected immediately before ("O"h), 24h and 72h after the start of the MTX infusion. The patients were on a normal diet, but without any fermented milk product. Normal values of D-lactate were determined in 14 healthy children admitted for elective surgery. Blood for the in vitro assay of glyoxalase activity was obtained from 4 healthy adult volunteers.

Description of materials and details of methodology can be found in the enclosed full paper in ANNEX (IX). To sum it up, the concentration of D-lactate was measured by end-point enzymatic assay with D-lactic dehydrogenase and fluorometric detection of reduced nicotinamide adenine dinucleotide. The activity of glyoxalase I enzyme was assayed by measuring the initial rate of formation of S-D-lactoylglutathione from the hemi-thioacetal in the presence of diluted erythrocyte lysate or yeast glyxolase; the reaction mixture contained reduced glutathione and methylglyoxal (see also Fig.12).

The plasma level of D-lactate was elevated ($P<0.02$) in the ALL patients before therapy compared with the healthy controls (Fig.13A). MTX induced a significant ($P<0.001$) fall in the D-lactate level, but at 72 h it was again close to the initial value (Fig.13B).

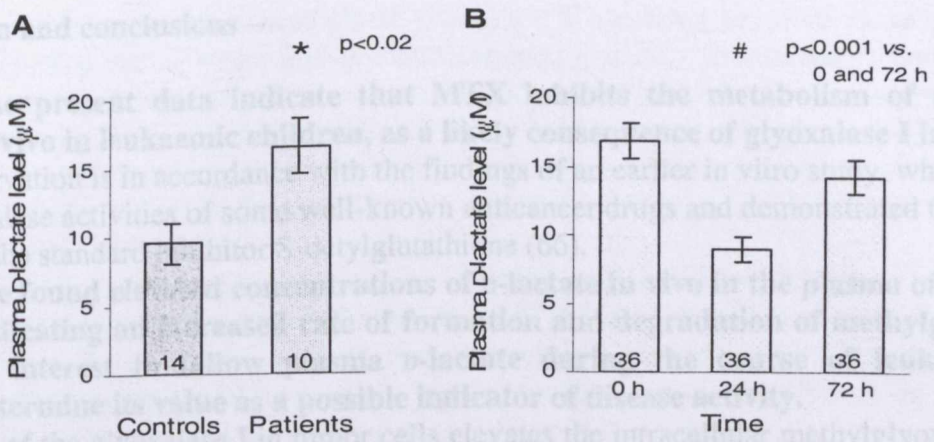

Fig.13 A;B A: patients with acute lymphoid leukaemia exhibit an elevated in vivo plasma D-lactate level (mean +/- standard error of the mean (SEM)). B: methotrexate induces a significant, transient fall in plasma D-lactate concentrations, as demonstrated by the data obtained from 36 treatment cycles (mean +/- SEM).

The glyoxalase I activity of human red blood cell lysates, and also the activity of yeast glyoxalase I were inhibited by all three folates (Table 3).

	methotrexate	folic acid	folinic acid
	μM	μM	μM
Haemolysate	125 +/- 4.6	112 +/- 5.8	221 +/- 9.6
Yeast glyoxalase	206 +/- 6.5	154 +/- 2.2	310 +/- 12.6

μM IC50, concentration causing 50% inhibition, SD, standard deviation.

Table 3 The IC50 values of glyoxalase I inhibition by folic acid and its analogues (mean +/- SD of four determinations)

MTX infusions which were followed by acute side-effects resulted in more pronounced changes in plasma D-lactate values: at 24 h P<0.05, at 724 P<0.001 (Fig.14.)

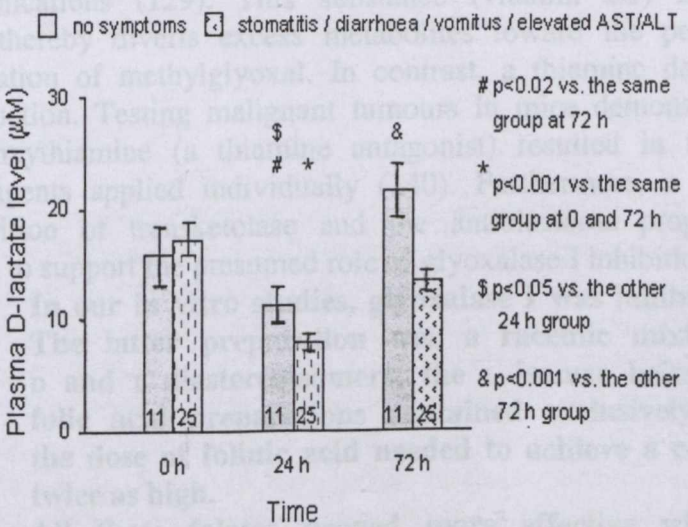

Fig14. The fall in plasma D-lactate level proved to be more pronounced when the methotrexate infusions were followed by the manifestation of acute toxic symptoms (mean +/- SEM).
AST, aspartate aminotransferase, ALT, alanine aminotransferase.

Discussion and conclusions

- **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.**

This observation is in accordance with the findings of an earlier in vitro study, which compared the antiglyoxalase activities of some well-known anticancer drugs and demonstrated that MTX was as potent as the standard inhibitor S-octylglutathione (66).

- **We found elevated concentrations of D-lactate in vivo in the plasma of leukaemic patients indicating an increased rate of formation and degradation of methylglyoxal. It would be of interest to follow plasma D-lactate during the course of leukaemia in order to determine its value as a possible indicator of disease activity.**

Inhibition of the glyoxalase I in tumor cells elevates the intracellular methylglyoxal level and induces apoptosis (128). The mechanism of the latter process is not fully understood, but a marked increase in the production of reactive oxygen species seems to be involved (5). Since the 1970s several glyoxalase inhibitors have been tested and proved to bear antitumor activity, both in vitro and in vivo (26, 128, 132, 139).

- **These data suggest that besides the inhibition of dihydrofolate reductase and thymidylate synthase, the glyoxalase I inhibitory property of MTX may contribute to the alnticancer and toxic actions of the drug.**
- **In the present study, the basal flux of metabolites through the glyoxalase system, as indicated by the plasma D-lactate, was not different before therapy between the patients with or without acute toxicities. By the end of the MTX infusion, however, the difference had become significant, and it was even more pronounced at 72 h. The lower D-lactate levels indicating a more expressed glyoxalase I inhibition in the event of toxic symptoms, support the role of an altered alpha-oxoaldehyde metabolism in the in vivo actions of the drug.**
- **This observation also indicated that MTX should prove more toxic in metabolic disorders involving an enhanced methylglyoxal production.**

In such disorders, the accumulation of toxic metabolites is accelerated also in non-cancer cells. Diabetes is a common disease in which the impaired glycolysis leads to enhanced formation of the alpha-oxoaldehyde, methylglyoxal (126). Indeed, diabetes proved to be the strongest predictor of MTX-induced lung injury in patients with rheumatoid arthritis (2).

The application of thiamine has been recommended for the prevention of diabetic complications (129). This substance (vitamin B1) is a stimulator of transketolase activity, and thereby diverts excess metabolites toward the pentose phosphate pathway, decreasing the formation of methylglyoxal. In contrast, a thiamine deficiency leads to enhanced methylglyoxal production. Testing malignant tumours in mice demonstrated that the combination of MTX with hydroxythiamine (a thiamine antagonist) resulted in higher antitumor efficacy compared with the agents applied individually (140). Furthermore, a close correlation was found between the inhibition of transketolase and the antiblastoma property of the preparation. These findings seem to support the presumed role of glyoxalase I inhibition in the actions of MTX.

- **In our in vitro studies, glyoxalase I was inhibited by MTX, folic acid and folinic acid. The latter preparation was a racemic mixture, containing equal amounts of the D and L diastereoisomers, the L isomer being the active form (76). The MTX and folic acid preparations contained exclusively active substances. This explains why the dose of folinic acid needed to achieve a comparable inhibition was approximately twice as high.**
- **All these folates proved more effective when tested on haemolysates compared with the isolated enzyme protein. This phenomenon may be related either to species differences (human vs. yeast enzyme) or the metabolism of folates and the formation of more active inhibitory derivatives by the red blood cells.**

The abnormal activity or expression of glyoxalase I has been reported in several types of human cancer, including colon, renal and prostate cancers, and also in leukaemic cells. Furthermore, this phenomenon has been identified as one of factors responsible for resistance to chemotherapy; accordingly, glyoxalase inhibitors have been found to be effective drug resistance-reversing compounds (among others 2-crotonyloxymethyl- 4,5,6-trihydroxycyclohex-2-enone, COTC, an earlier defined SH-inhibitor antitumor antibiotic (121) and glutathione analogues as well) (43, 50, 55, 106, 107, 121).

- **In the present study, patients were treated with relatively low dose (up to 60 mg/m^2/day) folinic acid in the 48h folloning the MTX infusion: plasma** D-**lactate levels gradually increased up to the end of the observation period, indicating that the tissue folate concentrations achieved were not sufficient for a significant in vivo inhibition of glyoxalase I.**

Folinic acid is frequently used in combination, e.g. with fluorouracyl in the therapy of colorectal cancer as it potentiates the inhibitory action of the latter drug on the enzyme thymidylate synthase (95). The glyoxalase I inhibitory action of folates provide a rationale for an investigation of folic acid and folinic acid in chemotherapy regimens with regard to their activity to alter resistance to anticancer agent-induced apoptosis.

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Finally, in connection with our studies concerning methylglyoxal and glyoxalase I, as an alumna of the University and of its predecessor in title:**Albert Szent-Gyorgyi,** Medical University, I would like to pay tribute here to our **Nobel Laureate Professor**. The "Submolecular theory of cancer" (besides extensive biochemical overviews on D-lactate and methylglyoxal metabolism by the same author) has been thoroughly discussed in "In memoriam Albert Szent-Gyorgyi", who analysed (forty years ego) the roles of **retin (methylglyoxal) and promin (glyoxalase I)**. The following conclusions could be drawn: the data currently at disposal are not sufficiently convincing either to verify or to confute the theory; and compounds able to influence the glyoxalase-system can be regarded as tumour-selective anticancer agents (54).

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Addendum

Our studies outlined in the previous three chapters dealing with different (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. As overviewed with a time lag of 19 years, an improved survival together with a better quality of life follow from the therapeutic research, and clinical trials involving the continued efforts of multidisciplinary, transdisciplinary teams and researchers in the laboratory and at the bedside (99, 134).

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ANNEX

Enclosed full papers	Publication No.
	I
	II
	IX