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Childhood leukaemia: therapeutic and experimental approach

By

D. SCHULER

Second Department of Paediatrics, Semmelweis University Medical School, Budapest

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A summary is given of some activities of the Hungarian Study Group for Childhood Leukaemia. Coordinated efforts within the Study Group led to improved therapeutic results. The median survival of patients diagnosed in 1971 was 12 months. For a comparison, out of the 57 patients diagnosed in 1978, 34 are still in their first remission. Experimental work designed to approach the pathomechanism of the disease provided the following main points of interest. *1.* Children diagnosed to have L_1 type ALL according to the FAB categories fare better than those with L_2. *2.* ADA activity was essentially normal in patients being in remission and their parents. *3.* Steroid receptor determinations can be of value in the planning of therapy. *4.* Very low as well as very high initial WBC count indicates a bad prognosis. *5.* Children who possess the DR 5 histocompatibility antigen have a better outlook for long complete remissions and cure. *6.* Growth hormone secretion and, accordingly, growth rate was normal in the long surviving patients. *7.* There was a slight impairment in the performance IQ of children who were under 6 years of age at the time of diagnosis. *8.* Emotional disturbances were universal in all patients, but could be much alleviated by regular psychological care and play activity. *9.* So far we observed one patient with a second malignancy: his AML manifested 4 years after the successful removal and chemo-radiotherapy of his brain tumour. *10.* Children in long, continuous remission — thought to be cured of their disease — possess normal remission lymphocytes with a significantly shorter cell cycle than controls.

Successful treatment of childhood leukaemia was a rarity before the late 1960s. With the introduction of new cytostatic drug combinations, therapeutic results showed gradual improvement and today some of the best treatment centres claim 75% long lasting remissions, with 40–50% of the children achieving a "cure" in ALL.

A survey, carried out in Hungary in 1969 by paediatricians, haematologists and oncologists, showed a rather dismal situation in the treatment of childhood leukaemia. It was those findings that prompted us to join forces and establish a Study Group in order to improve the situation. As from 1970, all children in Hungary suspected to have leukaemia were investigated, diagnosed and treated according to uniform principles, established at regular meetings of the Study Group. Cytological classification, cytochemical tests and clinical data handling is carried out in the Budapest Centre (National Institute of Paediatrics). Some of the most important clinical and therapeutic features of each patient are stored on magnetic tape and

are periodically assessed with the aid of a computer program. At present information is available on 740 leukaemic children diagnosed between 1970 and 1980. All cytostatic drugs are distributed — cost-free — by the Centre. Much as a result of this teamwork, therapeutic results showed considerable improvement since the establishment of the Study Group. In this paper I should like to outline some of the therapeutical results we have achieved as well as some of the research activities carried out in this field.

Methods

Cyto-morphological classification was done according to the FAB criteria [1]. Surface marker analysis entailed the use of E-rosettes, surface membrane immunoglobulins and in some of the cases anti-ALL serum. ADA determinations were carried out according to Kalckar [13]. TdT activity was measured according to the method of Hoffbrand [11B]. Standard microcytotoxicity was used in HLA and DR typing [25] in the institute of Haematology and Blood Transfusion. Steroid hormone receptor studies were carried out in the Institute of Biochemistry, as described by Munck and Leung [20]. Cytogenetic analysis was done by conventional methods and Giemsa banding [31] as well as Hoechst – Giemsa staining for SCE [24] Psychological assessment was through the Wechsler intelligence scale for children [36] and the World test [5, 2]. Therapeutical results were evaluated using life-table analysis and the Logrank test [25].

Results and Discussion

Meaningful evaluation of therapeutic approaches and results requires precise diagnosis and classification in leukaemia. This was attempted by morphological, immunological, enzyme and receptor studies.

The FAB classification proved to be the most reliable way for morphological categorization of the leukaemic cell types. In a detailed, retrospective analysis of bone marrow smears stored at the Centre, there was a 95% concordance rate between us when classifying these slides. The distribution of cases among the FAB categories is shown in Table I. The two major categories within the lymphoid group are L_1 and L_2. We demonstrated for the first time that patients with L_1 morphology fare better than those with L_2 [14]. This finding indicated a place among the prognostic criteria for the morphological typing of the presentation cells and has since been confirmed by a number of centres abroad. Cytochemical reactions, however, are still of great value in the differential diagnosis of leukaemia, mainly in the differentiation between the immature forms such as L_2 and M_1.

Of the immunological markers, T and B cell determinations are the most widely used. A drawback in their use is the need to work with cell suspensions which makes multicentre studies difficult. The results in Table II therefore represent only some of our patients. The most frequent form of childhood ALL shows no T or B cell differentiation and gives a positive reaction with anti-ALL serum. B cell or Burkitt cell leukaemia is very rare in children (see Table II). Recently, we carried out a detailed analysis on two patients with this form of ALL [32].

TABLE I

Morphological diagnosis in childhood leukaemia according to FAB categories

FAB type	L_1	L_2	L_3	M_1	M_2	M_3	M_4	M_{5A}	M_{5B}
No. of patients	286	155	9	25	17	2	25	8	4
per cent distribution	53	29	2	5	3	0	5	2	1

TABLE II

Inmunological markers in the patients

No.	E-rosette	SmIg
12	+	—
2	—	+
25	—	—

TABLE III

ADA activity in leukaemic children and in their parents

Diagnosis	No. of cases	ADA U/10^7 cells	
		mean S.D.	range
Controls	10	1.9 ± 0.38	1.2—2.4
ALL	8	14.3 ± 9.78	4.0—30.0
ALL in relapse	5	7.8 ± 5.20	4.8—17.1
ALL in remission	10	1.8 ± 0.83	1.0—3.7
parents	10	2.2 ± 0.18	2.0—2.5

B cell leukaemias show an unfavourable course, with early relapse and death. Recently, other markers have acquired a major role in the classification of acute leukaemias, the most widely used ones being monoclonal antibodies [12, 27].

Enzyme markers have been introduced in leukaemia diagnosis some 6—8 years ago. Terminal transferase, being the most widely used, was determined in some of our patients. Although numbers are still low, we could demonstrate the activity of this enzyme in lymphoid leukaemias [16].

ADA determinations were carried out in leukaemic patients and their parents. Previous studies found an increased activity of this enzyme among parents of leukaemic children [35], a finding that could have important genetic implications. We were, however, unable to confirm this observation and found normal values in

both patients and parents (Table III), [15].

Prednisolone is one of the most effective drugs in leukaemia treatment. Some patients, however, react to it better than others. In collaboration with the 2nd Institute of Biochemistry, we carried out investigations on the steroid hormone receptors of leukaemic cells. The common, non-T non-B ALL cells contained high numbers of steroid receptors and responded well to steroids both in vitro and in vivo [22]. Somewhat surprisingly, at times other leukaemic cell types are also found to have steroid receptors. We found e.g. high numbers in one case of AML and one case of CML. These experiments provide useful information on the drug sensitivity or resistance of certain blast-cell populations and may eventually prove essential in the planning of therapy.

Clinical experience gained in the last decade shows that the cure rate in acute leukaemia is closely related to the intensity of the initial phase of treatment. More aggressive treatment leads to more complications and some iatrogenic deaths. It is therefore important to group the patients according to prognostic criteria and to administer more intensive treatment only to those who are likely to have a very unfavourable prognosis. Some of the widely used and easily recognizable prognostic features are shown in Table IV. Of these, high initial WBC count and hepato-splenomegaly are thought to be most important. In a retrospective study we found that patients with very low WBC counts (less than 1 G/l) also do badly, which may indicate the necessity of including this criterion in the bad prognostic group. Sex is not a per se prognostic feature, although boys seem to have some 20% less chance of cure than girls. This is mainly because of the problem of testicular relapse, which is always followed by systemic relapse of the disease. According to Moe, this complication can be prevented by the use of intermediate dose methotrexate following the induction phase [18]. Our studies along these lines are currently being pursued.

Using the same treatment, thought to be optimal at present, some children relapse and die, while others carry on in remission and are for all practical purposes cured. It has been suggested that the immune system of the patients may have an important

TABLE IV

Prognostic factors in ALL patients not yet in remission after 4 weeks treatment

	High risk patients	Low risk patients
Initial WBC	> 50 G/l	< 50 G/l
Mediastinal mass	+	—
Initial meningeal infiltration	+	—
Age	<1 year, >12 years	1—12 years
T-cell marker	E rosette positive	—

role in eliminating residual leukaemic cells. Immune response genes (Ir) are closely located to those of the tissue antigens coding for the expression of HLA and DR phenotypes. It is somewhat surprising therefore that there are relatively few studies on the HLA distribution of long-surviving leukaemic patients. A number of authors found a higher incidence of HLA-A_2B_{12} and HLA-A_2B_{40} haplotypes among these patients [9, 3, 4, 28] while others found an association between HLA-A_9, HLA-$B_{17}AW_{33}$ and long survival [17, 7, 34].

In our studies the data of 37 long surviving children and 13 short term survivors were analysed. We found no association between survival and any of the HLA haplotypes. In the last two years typing is also possible for the D locus of the HLA complex. So far only one incomplete examination [6] has been published on the DR distribution of leukaemic patients. We found a strong correlation between DR 5 antigen and the long continuous remission of children with ALL. As compared to the 20.2% DR 5 frequency among the controls, long-term survivors showed a 40.5% positivity for this antigen. The finding seems to indicate an important prognostic value of DR typing. Further, it may have important implications in the search for the aetiology of the disease.

Therapeutic results in leukaemia — as in other potentially fatal diseases — can best be assessed through life-table (survival) analysis. A considerable number of patients are lost early and about 35—40% relapse in the first 3 years of therapy. Therapy is discontinued after 3 years, and during the following year there is again an increased number of relapses and deaths. Figure 1 shows the survival curves of patients diagnosed in different years. There are very few long term survivors in the group diagnosed in 1971. Two years later (1973) results were much improved and it is hoped that patients on current therapy will achieve even better results. It can observed on the curves that after 3—4 years, survival curves form a plateau which means that after a time very few relapses occur. The number of patients surviving for 4 years or more is 50 at present. The current treatment protocols are shown in Figs 2a and b.

The increasing potential for cure made it mandatory to study the late effects of therapy. We investigated the hormonal, cytogenetic and psychological late effects of preventive CNS irradiation. Some authors claimed diminished growth hormone secretion following skull irradiation [8, 10, 33], while others failed to confirm these findings [21, 19]. In our own series, normal levels of growth, thyreotrop and adrenocorticotrop hormones were demonstrated. Accordingly, we found no growth retardation among 40 leukaemic children during a minimum of 3 years following the start of chemotherapy [30A].

Intelligence testing revealed a slight impairment in performance among the children who were less than 6 years old at the time of diagnosis [29]. These findings are in accordance with those

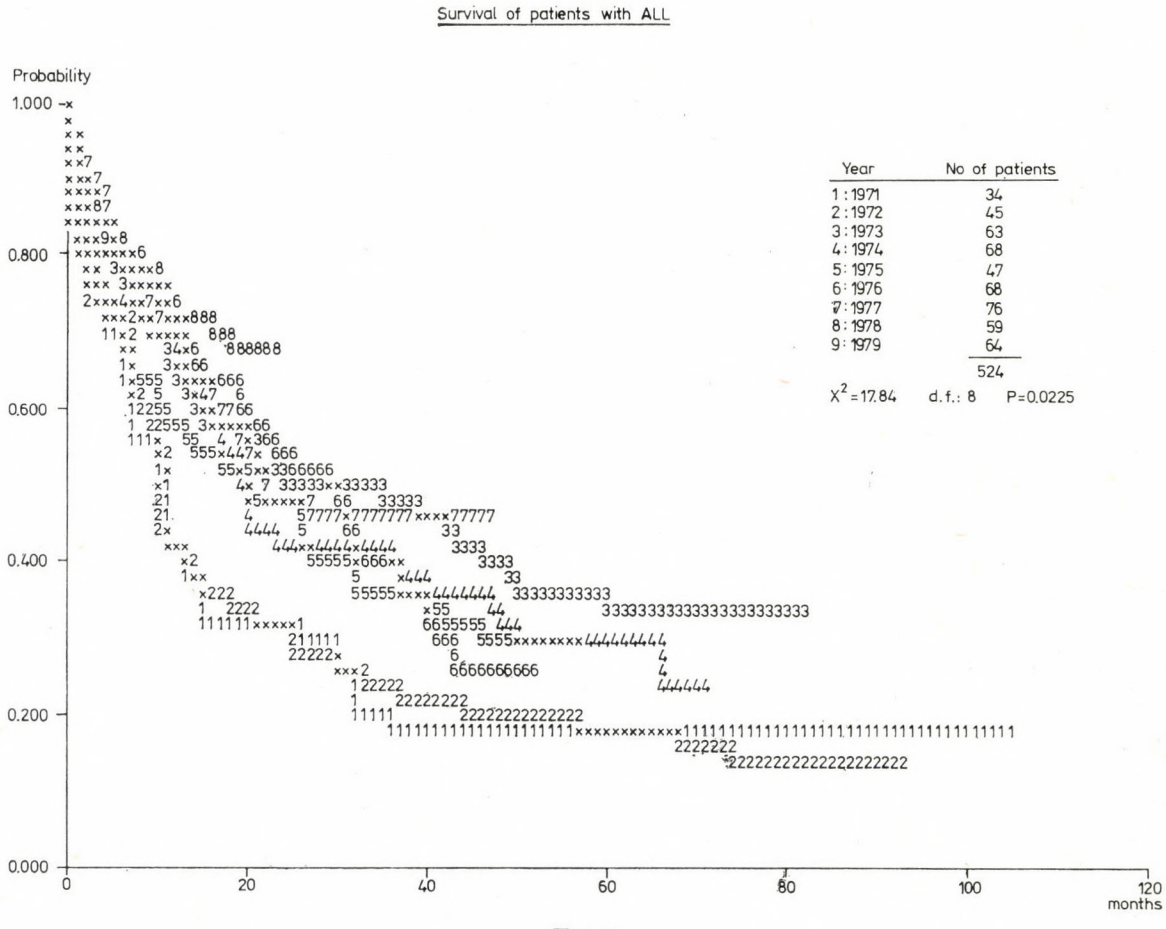
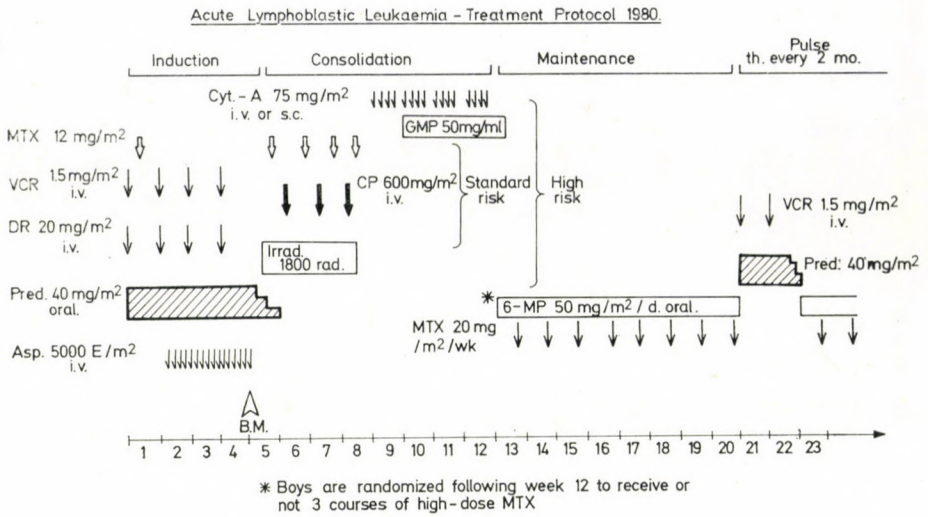
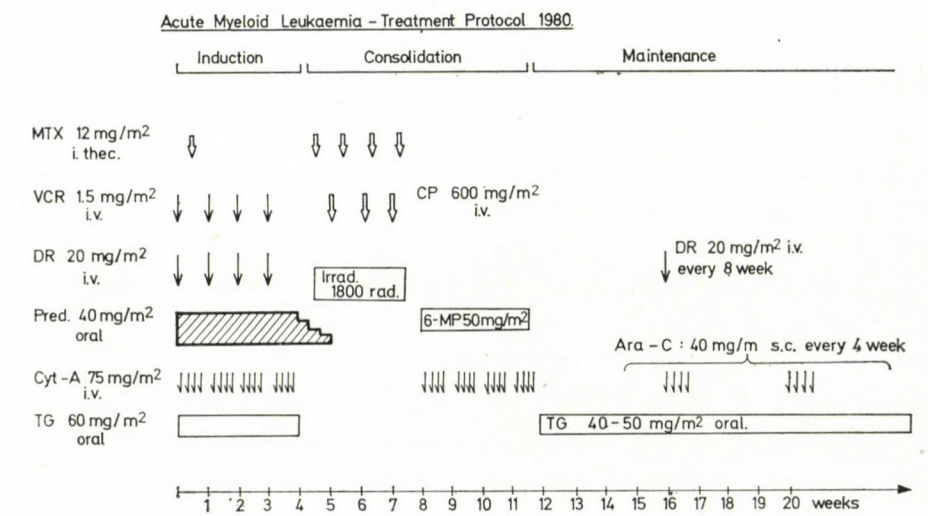

Survival of patients with ALL
Probability
1.000
0.800
0.600
0.400
0.200
0.000
0
20
40
60
80
100
120
months
Year
No of patients
1 : 1971 34
2 : 1972 45
3 : 1973 63
4 : 1974 68
5 : 1975 47
6 : 1976 68
7 : 1977 76
8 : 1978 59
9 : 1979 64
524
X² = 17.84 d.f.: 8 P=0.0225

FIG. 1





FIGS 2a, b

of Eiser and Lansdown [11A]. Detailed psychological testing showed that practically all the leukaemic children had some type of emotional disturbance. Regular psychotherapy and play activity greatly reduced the incidence and intensity of these problems (Table V). Creative activity proved by far the most effective of the measures in reducing emotional disturbances. We think it imperative to offer psychotherapy to parents too; it can be either individual, or group therapy. Because of the considerable

TABLE V

The effect of psychological care in per cent of total number of patients as indicated by the World test

Patient groups		Number of cases	Anxiety	Disturbed self-image	Slowing of motorium	Fear of death	Isolation
Leukaemia	No psychological care	25	100	64	64	92	92
	Psychological care	23	30	17	30	17	17
Control		24	21	0	8	0	21

strain on the staff in the oncology wards, doctors and nurses should also take part in psychotherapy sessions.

Of the late effects more and more recognition is given to secondary malignant tumours. These, according to the literature, occur in 5—10% of the succeessfully treated first tumours. So far we observed only 1 such patient; his first malignancy was however a solid tumour. He developed acute myeloid leukaemia 4 years after the removal and chemo-radiotherapy of his brain ependinoma.

Because of the 3-year cytotoxic therapy with all its possible mutagenic effects, cytogenetic studies are of great interest in the follow-up of leukaemic patients, considered to be cured of their disease. We studied 27 of our patients, who having completed 3 years on chemotherapy were off treatment and relapse-free at the time of the investigation. Both conventional and ASG banding techniques were employed in these studies. The frequency of aberrations (deletions and acentric fragments) was the same as in the controls. Sister chromatid exchange (SCE) is generally considered to be the most sensitive method for the demonstration of chromosomal mutations. Previously there have been very few long surviving leukaemic children investigated with this approach [23]. In a recent work with Szollár, we studied 27 children in long continuous remission and found no increase in the frequency of SCE-s. Through BrdU incubation one can get a good assessment of the probable cell cycle time of cell populations, as cells in the 2nd, 3rd or 4th cycle show considerable differences in BrdU uptake. With this approach we studied for the first time this problem in long surviving leukaemic children, and found significantly less cells in the 2nd cycle and more in the 3rd cycle after 72 hours BrdU incubation in the patients than in the controls. [30B]. This points to a shorter cell cycle in the patients than the control value. So far nothing abnormal or unusual has been reported in the remission lymphocytes of leukaemic patients, already off all chemotherapy and considered to be cured. It is still an open question whether the presence of cell populations with a shorter cycle time

is the consequence of the long-term cytostatic treatment, or whether it existed before the diagnosis was made. If the former is true we could suppose a selective advantage for the cells with a shorter cycle, during chemotherapy. If, however, the later possibility is found to be true it could perhaps mean a predisposition to leukaemia with short cycle cells. Studies are being done to elucidate this problem.

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Prof. D. Schuler
Tűzoltó u. 7
1094 Budapest, Hungary