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Lymphocyte markers in childhood ALL: The lack of correlation with prognosis

E. Cristo Morón, T. Révész, D. Schuler

Department of Paediatrics, C. J. Finlay Hospital, Habana, Cuba, and Second Department of Paediatrics, Semmelweis University Medical School, Budapest

Presentation blast cells from 39 children with acute lymphoblastic leukaemia were studied for T- or B-lymphocyte characteristics. Eleven patients had blasts with T-cell phenotype and one child had B-cell leukaemia. The rest belonged to the non-T, non-B group. Clinical features were similar in the T-cell and non-T, non-B cell group, including the initial WBC count and the presence of mediastinal mass. Although patients with T-cell leukaemia fared worse than those in the non-T, non-B group, the difference in survival was not statistically significant. These data are not in accordance with the findings of most other groups and are thought to stem from the chance occurrence of relatively low initial WBC counts in the T-cell group.

Twenty years ago childhood leukaemia almost invariably had a fatal outcome. With the introduction of effective cytostatic drugs an increasing proportion of patients achieved full remission. Combination chemotherapy and preventive central nervous system irradiation have led to the promising current situation, which means that 90–95% of the children achieve complete remission. Although 30–40% relapse while still on maintenance therapy, 50–60% of the patients continue in first remission. Some of the best centres report on a 50–65% 5 year relapse-free survival in their ALL patients.

Improving therapeutic results, however, impose the question why some patients relapse early in the course of their disease. The recognition of initial prognostic factors has been a focal point of interest during the past decade. The list of factors known to be associated with a poor clinical response is quite considerable. Some of these prognostic features are listed in Table I.

As the great majority of childhood leukaemias is of lymphoid origin, the introduction of lymphocyte markers in the 1970-s was almost instantly followed by marker studies in ALL. Leukaemic blasts showing T-lymphocyte features characterized a subset of the leukaemias (1). It was recognized early that patients with T-cell leukaemia tend to have

Abbreviations

ALL: acute lymphoblastic leukaemia
WBC: white blood cell
CNS: central nervous system

TABLE I

Factors indicating poor prognosis in ALL

High WBC count (over 50 G/l)
Massive organomegaly
Mediastinal mass
Initial CNS involvement
Male sex
Age: less than 2 years
over 12 years?
T-cell character?
B-cell character
Normal initial Hgb
Black race

a high initial WBC count, mediastinal mass, hepatosplenomegaly and are more often boys. T-cell ALL constitutes some 20% of the acute lymphoid leukaemias.

ALL blasts sometimes derive from the B-lymphocyte axis. These cells carry membrane immunoglobulins, and often present with a characteristic morphologic picture called Burkitt-cell leukaemia (3).

Recently other membrane markers have been introduced in the diagnosis of childhood leukaemia. Some of these define T- or B-lymphocyte subclasses and thus indicate the level of maturation at which the leukaemic cells are 'frozen'. Others demonstrate early fetal antigens that, by virtue of their expression on certain leukaemias, point to the haemopoietic origin of the leukaemic process.

In this paper we analyse the clinical data of 39 children with ALL, whose blasts at presentation were examined for T- and B-lymphocyte markers. Contrary to most literary data we failed to demonstrate a truly significant difference between the patients with T-cell, and those with non-T, non-B ALL.

PATIENTS

Thirty-nine children with previously untreated ALL were analysed. They were diagnosed between 1975 and 1980, and were followed up for 9 to 57 months at the time of analysis. Diagnosis was based on bone-marrow examinations. Smears were stained with May–Grunwald–Giemsa. The FAB criteria were used when establishing the morphological diagnosis. PAS and Sudan-black cytochemical reactions were used routinely and if necessary, complemented by non-specific esterase reactions.

ALL patients had chest X-rays at the time of admission. Hepato-splenomegaly was considered severe, when one of these organs was palpable below the umbilical line. Patients were treated according to uniform protocols. For remission induction, vincristine, daunorubicin and prednisolone were used and in about half of the cases asparaginase. CNS prophylaxis consisted of cranial irradiation and intrathecal methotrexate. 6-mercaptopurine and methotrexate were used for maintenance

chemotherapy. Treatment was continued for a total of 3 years and stopped thereafter, provided that the child was still in full remission.

Methods

Peripheral blood mononuclear cells were separated by simple or density gradient centrifugation (2). T-cells were counted on the basis of their capacity to form E-rosettes as described by Jondal et al. (7). B-cells were identified by immunofluorescence using polyvalent rabbit anti-human Ig antibody. Cells were pre-incubated at 37 °C for 30 min prior to staining in order to eliminate cytophilic immunoglobulins (12). In each technique, 200 cells were counted. In some cases anti-T and anti-B lymphocyte sera were used for checking the results obtained with the above methods. The single case with B-type blasts was analysed in more detail as to the nature of the immunoglobulin on the cells' surface.

A patient was considered to have T- or B-cell disease if 30% or more of the blasts were E-rosette or Ig positive, otherwise the case was classified as non-T, non-B.

Remission and survival data of the patients was analysed using the logrank test (10).

Results

Surface marker studies indicated a T-cell origin in 11 of the 39 newly diagnosed ALL patients (28%). One child presented with immunoglobulin-bearing blasts, while the rest of the patients (69%) had non-T, non-B ALL. Morphological analysis of the presentation bone-marrow smears showed a typical 'Burkitt-like' picture (L_3) in the child with B-cell disease. All the other patients had L_1 or L_2 morphology and there was no difference in the distribution of L_1-s and L_2-s between the T-cell and the non-T, non-B group.

Table II

Initial clinical features in childhood ALL

	Non-T Non-B ALL	T-ALL	B-ALL
No. of patients	27	11	1
Median age, years	5.0	5.5	12.5
Male/female ratio	1.3:1	10:1	Male
Hepatomegaly	13	7	0
Splenomegaly	15	7	0
Lymphadenopathy	13	8	1
Mediastinal mass	1	1	0
Initial WBC count $\leq$ 50 G/l	24	8	1
$>$ 50 G/l	3	3	0
Initial Hb $\leq$ 4.0 mmol/l	14	4	
$\geq$ 4.0 mmol/l	13	7	1

Initial clinical features of the patients are shown in Table II. Median age and the extent of organomegaly were approximately the same in the T and non-T groups. In contrast with data in the literature, the initial WBC count was elevated only in a quarter of the patients with T-ALL. The ratio of boys to girls, however, was much higher in this group.

All patients in the T-cell group achieved remission, while one child in the non-T, non-B group, and the child with B-ALL failed to remit. Analysis of the response to therapy was carried out using the life-table method and logrank analysis. Although patients with T-ALL had a more steeply declining survival curve, the difference between the two groups was not significant (Fig. 1). Analysis of the remission curves (Fig. 2) showed the T-cell group to have inferior results but the difference — at the time of analysis — was not significant statistically.

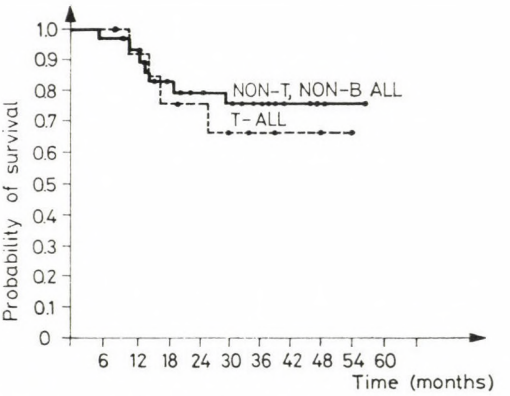

FIG. 1. Survival in ALL, according to cell-surface characteristics. No. of patients: Non-T, non-B ALL: 27, T-ALL: 11, $\chi^2 = 0.28$, $P > 0.1$

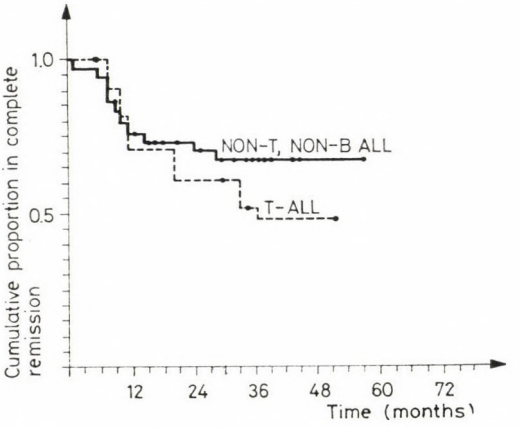

FIG. 2. Cumulative complete remission in ALL according to cell surface characteristics. No. of patients: Non-T, non-B ALL: 27, T-ALL: 11, $\chi^2 = 0.785$, $P > 0.1$

TABLE III

Rate and site of relapse in ALL

	Non-T, non-B ALL	T-ALL
No. of patients	27	11
Site of first relapse:		
Bone marrow	6	3
Meninges	2	2
Testes	—	2
Combined	1	—

The patient with B-cell ALL did not achieve remission and died while still in relapse after 2 months of treatment.

The relapse rate seemed to be higher in the T-cell group (Table III) and although 2nd and 3rd remissions could be achieved, they were short-lived.

DISCUSSION

The rapid development of immunological methods during the past decade expanded the field where they can be employed for the detection of pathological processes. Lymphoid leukaemias were among the first malignancies to be characterized by immunological approaches. The analysis of cell surface antigens and other biochemical characteristics provides a phenotypic pattern unique for the given cell proliferation (4). T and B-cell markers allowed the identification of the target cell of the leukaemic process in 20–25% of the childhood leukaemia cases.

In our material 28% of the patients presented with T-cell leukaemia, i.e. more than 30% of the blasts formed E-rosettes and stained positively with anti-T-cell serum. These patients were reported to have a worse prognosis with high initial WBC count, expressed organomegaly and mediastinal mass. We found the same tendency but have failed to demonstrate a significant difference in these factors between the T-cell group and that of the non-T, non-B variety (Table II). Analysis of their remission and survival curves showed the T-cell group to fare worse, but the difference at present is not significant statistically. Recently it has been suggested that the worse prognosis in the T-cell group is not linked to the cells' surface phenotype but simply to the higher initial WBC in this group (9). If patients with T-cell leukaemia are analysed according to their initial leukocyte count, those with lower counts fare equal to the non-T, non-B group. The numbers in our series do not allow for such an analysis, especially since

only 3 of the 11 patients with T-cell disease had WBC counts over 50 G/1. This seems to be the reason why we failed to show a statistically significant difference between the two groups at the time of analysis. The long-term results, however, are most likely to be inferior in the T-cell group, since these patients had more relapses than those in the other group (Table II).

The single patient with B-cell leukaemia was found to have blasts which all carried a monoclonal immunoglobulin on their surface. After trypsinization nearly all the cells resynthetized the antibody molecules. This patient did not achieve remission and in spite of intensive chemotherapy died 2 months after the diagnosis. B-cell leukaemia seems to be a particularly aggressive proliferation and its treatment remains completely unsolved (8).

Lymphoid leukaemias can further be characterized by the use of xenogeneic antisera directed against 'leukaemia-associated antigens'. Greaves et al. (5) were the first to demonstrate the presence of the so-called common-ALL antigen (cALLa) on the majority of ALL cells. This antigen, however, may very well turn out to be an early differentiation antigen, normally found on early haemoprecursors. Nevertheless, this antibody proved to be an operationally most useful reagent not only in differential diagnosis but also in throwing light on the association of various leukaemias (6).

The latest development in immunodiagnosis employs a wide variety of monoclonal antibodies. Reinherz and Schlossman (11) produced a series of anti-T cell antibodies that characterize T-cells in different stages of development and an antibody seemingly detecting the same cALL antigen as Greaves' xenogeneic antisera (12). These antibodies can be produced in bulk which makes them more accessible for diagnostic purposes and should provide valuable information in the next few years concerning the origin and development of various leukaemias.

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E. Cristo-Morón, M. D.
Tűzoltó u. 7
H-1094 Budapest, Hungary