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A three-year prospective study of 137 cases of acute leukaemia in Zimbabwe

B PAUL, J M MUKIIBI, A MANDISODZA, L LEVY and F K NKRUMAH

SUMMARY

We studied 137 cases of acute leukaemia seen between December 1985 and November 1988, using traditional staining techniques together with cytochemistry and in cases of probable acute lymphoblastic leukaemia (Sudan Black negative) by immunophenotyping. Not all tests were carried out in every case (some cases of ALL could only be classified as T or non-T).

Paediatric group (age≤14 yrs): 75 cases — acute lymphoblastic leukaemia 52, acute myeloid leukaemia 18, acute undifferentiated leukaemia 5. Peak incidence in 5–9 year group. Male:Female ratio = 1,7:1. acute myeloid leukaemia was associated with chloromas in 2 cases (11 pc).

Adult group: 62 cases — acute lymphoblastic leukaemia 23, acute myeloid leukaemia 36 and acute undifferentiated leukaemia 3. Peak incidence in 50–54 age group. Male:Female ratio = 1:1,2.

Acute lymphoblastic leukaemia subtypes (all ages) T 16, Common 20, Null 12, 'non-T' 16, B cell 0, untyped 11. 69 pc were of L2 morphology. In T-ALL, 11 had thymomas and Male:Female ratio = 15:1. Male:Female ratio for 'non-T' = 1,5:1.

Acute myeloid leukaemia subtypes (all ages) M1 3, M2 8, M3 14, M4 19, M5 8, M6 2, M7 1.

Overall incidence of acute leukaemia appears increased at 0,91 per 100 000 per annum from previous studies in Zimbabwe. Common ALL (mean age = 13 years) is an emerging problem and now outnumbers T-ALL (mean age = 10 years). This may be related to a general improvement in living standards and health in Zimbabwe.

INTRODUCTION

There is a general paucity of information on the epidemiology of acute leukaemia in Africa especially since the explosion of knowledge in cell differentiation that has occurred over the past few years — a bibliography was recently published.[1] An accurate diagnosis is essential if these disorders are going to be treated aggressively, and also classification relates to prognosis. The pattern of acute leukaemia may be changing in some parts of Africa and this may give some aetiological clues.[2]

Many studies have been retrospective surveys of hospital records[3,4,5] and most were before the introduction of cell marker studies. We have carried out a prospective study using traditional staining techniques together with cytochemistry and immunophenotyping.

MATERIALS AND METHODS

In the period December 1985 to November 1988 inclusive, 137 cases of acute leukaemia were diagnosed in patients of all age groups: 129 cases came under our direct care, and a further eight cases were diagnosed on blood and marrow films sent from elsewhere (four from Mpilo Hospital (Bulawayo) and four from other hospitals).

Morphological classification was based on the French-American-British system by examining Giemsa stained blood and marrow smears.[6,7,8] In the case of acute lymphoblastic leukaemia a scoring system was used to differentiate between L1, L2 and L3 morphology.[7]

Further studies carried out included Sudan Black B and Periodic Acid Schiff stains by routine methods.[9] Myeloperoxidase, chloroacetate esterase, α-naphthyl acetate esterase, and acid phosphatase were carried out by commercial kits following the manufacturer's instructions (Sigma, St Louis, USA). Immunophenotyping included terminal deoxynucleotidyl transferase by indirect immunofluorescence (polyclonal antibody, SeraLab, Crawley, UK), and sheep E rosettes using fresh

Department of Haematology, Medicine and Paediatrics
University of Zimbabwe School of Medicine
P O Box A178
Avondale, Harare, Zimbabwe
Correspondence
B Paul
Dept of Haematology
Victoria Hospital
Workshop
Notts S80 2BN
UK

Figure 1: Incidence of acute leukaemia

Incidence of Acute Leukaemia

Rate / 100,000 per year

Age Group (Quinquennia)
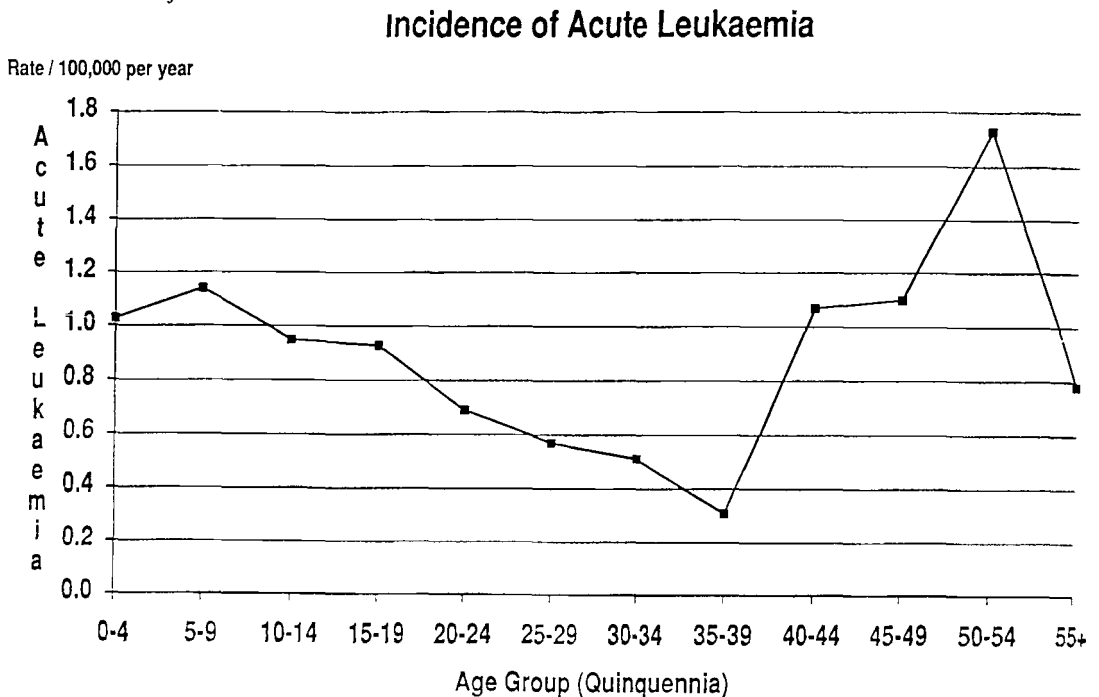
untreated washed red cells from African sheep of the university flock by a standard technique.[10]

Monoclonal antibodies (Ortho, Raritan, USA) were used by either indirect immunofluorescent or indirect immunoperoxidase techniques (Ortho) with the following specificities CD 3 (OKT3), CD10 (OK-CALLa), CD24 (OKB2), HLA class II (OKDR) and CD13 (UCHM1). Some tests were carried on cells in suspension after Ficoll separation or on direct smears if little material was available together with appropriate controls. Polyclonal antikappa, lambda, and mu (SeraLab) was used to detect surface or cytoplasic immunoglobulin by direct immunofluorescent techniques.[10] A result was considered positive if greater than 10 pc of blast cells reacted.

Not all tests were carried out on each patient to conserve expensive reagents — where leukaemic cells had obvious Sudan Black positivity immunophenotyping was not carried out. Some cases of acute lymphoblastic leukaemia could only be classified as T cell (Sudan Black negative, Sheep E Rosette positive, localised Acid Phosphatase positive), or non-T cell type (Sudan Black negative,

Sheep E Rosette negative, diffuse or negative Acid Phosphatase).

M6 subtype was diagnosed by PAS positivity in the erythroblasts and M7 by PAS reaction and reticulin staining of the trephine biopsy.

Incidence rates are based on the sex and age distribution of the whole population (1982 census) and that the catchment population for Parirenyatwa Hospital at the time of the study was five million. Student's 't' test and chi squared tests were used for statistical analysis. White cell counts were log transformed for analysis.

RESULTS

During the three-year study period, 137 cases of acute leukaemia were seen: 75 (55 pc) in the paediatric group (aged 14 years or less) and 62 (45 pc) in the adult group (aged 15 years or more). This gives an overall incidence of acute leukaemia of approximately 0,9 per 100 000 per annum. The pattern based on quinquennial groups is shown in Figure I. Two peaks are seen at the age of 5-9 and 50-54 years with incidence rats of 1,14 and 1,73 per 100 000 per annum respectively.

Table I: Season variation of acute leukaemia

	Nov-Jan	Feb-Apr	May-Jul	Aug-Oct
ALL	21	22	14	21
AL	36	34	33	34

ALL = Acute lymphoblastic leukaemia
AL = Acute leukaemia (all types)

The month of presentation is shown in Table I. Data have been collected into three-month periods as the numbers in any one month are low. November–April cover the rainy season and May–October the dry season in Zimbabwe. Fewer cases of acute lymphoblastic leukaemia were seen in the May–July period, but this is not a significant difference (X^2 = 2,1, p >0,5).

Acute lymphoblastic leukaemia was the commonest type (69 pc) in the paediatric group (Table II). Two of 18 cases of acute myeloid leukaemia were associated with an orbital chloroma. Acute myeloid leukaemia was the commonest type in adults (58 pc) [Table III].

Table II: Subtypes of paediatric acute leukaemia

	M	F	M:F ratio	Total (pc)	
ALL	36	16	2,3:1*	52	(69)
AML	9¶	9	1:1	18	(24)
AUL	2	3	1:1,5	5	(7)
Total	47	28	1,7:1**	75	(100)

¶ 2 chloroma   *X^2 = 7,65 p<0,01
**X^2 = 4,81 p<0,05

Table III: Subtypes of adult acute leukaemia

	M	F	M:F ratio	Total (pc)	
ALL	14	9	1,6:1	23	(37)
AML	12	24	1:2*	36	(58)
AUL	2	1	2:1	3	(5)
Total	28	34	1:1,2**	62	(100)

* X^2 = 4,0 p<0,05   ** X^2 = 1,08 p = NS

Commonest types of acute myeloid leukaemia (Table IV) were myelomonocytic (35 pc) and promyelocytic leukaemia (25 pc) with 8/14 being the hypogranular varient (M3b).

Table V shows the subtypes of acute lymphoblastic leukaemia. Common ALL was the most frequent followed by T cell disease. Most cases of acute lymphoblastic leukaemia had L2 morphology (Table

VI). Most (69 pc) of T-ALL cases were associated with a large anterior mediastinal mass on chest X-ray and there is a marked male predominance in this group.

Table IV: Subtypes of acute myeloid leukaemia (all age groups)

Type	n	pc	WBC	(SD)
M1	3	5	44,8	(32,8)
M2	8	15	42,2	(46,5)
M3*	14	25	56,1	(67,9)
M4	19	35	85,1	(148,6)
m5¶	8	15	124,1	(202,2)
M6	2	4	35.9	—
M7	1	2	10,5	—

*M3a = 6   M3b = 8
¶M5a = 6   M5b = 2
WBC = at presentation x 10^9/1 (SD)

Table V: Subtypes of ALL (all age groups)

	n	pc	M:F	mean WBC	(SD)
T cell*	16	21	15:1	129,1	(205,0)
Common	20	27	1:1	56,8	(110,8)
Null	12	16	3:1	149,0	(261,5)
Non-T	16	21	1:1,3	84,7	(124,5)
Untyped	11	15	1,8:1	141,9	(246,9)
B cell	0	0			

* 11 (69 pc) associated with thymoma on CXR
WBC T v CALL t = 3,44 p = <0,01
WBC T v Null t = 2,79 p<0,01
WBC Null v CALL t = 7,68 p = <0,001

Table VI: Morphology and PAS reaction of ALL (all age groups)

L2 = 23 (31 PC)	PAS pos = 27 (37 pc)
L2 = 52 (69 pc)	PAS ng = 46 (63 pc)
L3 - 0	Not done = 2

Sixty three per cent of acute lymphoblastic leukaemia cases were PAS negative, though 16 of 20 (80 pc) of common ALL were PAS positive. Thus if PAS positive cases are considered as probable common ALL and are added to the known common ALL cases, the proportion of common ALL rises to 37 pc. The sex ratio is equal in the Common ALL group.

There was a small group of undifferentiated leukaemia (no myeloid or lymphoid markers on cytochemistry and immunophenotyping), with all

having a L2 morphology. Mean presentation WBC 87,9 x 10^9/1; SD 103,8 (n = 7).

DISCUSSION

Previous studies of acute leukaemia have been reported from Zimbabwe (formerly Rhodesia). Lowe's study covered a 5-year period (1967–72) — 46 cases were seen (acute lymphoblastic leukaemia 14, acute myeloid leukaemia 21, acute undifferentiated leukaemia 11) giving an overall incidence of about 0,3/100,000/year which is much lower than the present study (0,91).[4] For the period 1980–82, 63 cases of acute leukaemia were seen (acute lymphoblastic leukaemia 14, acute myeloid leukaemia 49) though some paediatric cases were not included.[11] This study showed a male predominance of acute lymphoblastic leukaemia (2:1) similar to the present series, but classification by subtype was not reported — overall incidence of acute leukaemia of 0,7/100 000/year was again lower than our current survey.

The increasing incidence may in part reflect better diagnostic facilities, but there is probably a true increase in the incidence of acute leukaemia in Zimbabwe as noted in other countries.[12] These Zimbabwean studies are not true population based surveys and thus the absolute figures must be treated with caution.

The incidence of acute leukaemia is still low in comparison with the United Kingdom (eg age 0–4 acute lymphoblastic leukaemia = 5,0, acute myeloid leukaemia = 0,8; age 55–65 acute lymphoblastic leukaemia = 0,7, acute myeloid leukaemia = 5,7.100 000/year).[13,14] Lower rates of acute lymphoblastic leukaemia are noted in American blacks, Jamaicans and Nigerians.[12,15]

Environmental factors may be important in the aetiology of some types of acute leukaemia.[2] We examined the seasonal incidence by month of presentation to see if this gives any clues. However, there is no significant trend in our patients despite a slight fall in part of the dry season (May–July) nor in the subtypes of acute lymphoblastic leukaemia.

All the major FAB subtypes of acute myeloid leukaemia were identified with M4 being the most common (35 pc). We were surprised at the number of M3 (25 pc) of which 8/14 were the hypogranular varient. Previous studies have shown marked

variation in distribution which in part may reflect lack of reproducibility of some FAB criteria — M3 averages 10 pc of acute myeloid leukaemia.[16] All our cases were classified by one author (BP) and M3 has characteristic morphology. A previous study in Zimbabwe also gave a relatively high incidence of M3 — 4/17 (24 pc).[4] Many patients presented with high blast cell counts and these were highest in the M5 group (mean 124 x 10^9/1).

Two boys presented with an orbital chloroma with M3 and M4 AML — a 10 pc incidence of chloroma in childhood acute myeloid leukaemia. A higher incidence of chloroma has been reported from West Africa (–50 pc),[17] East Africa (–23 pc),[5] and in South African blacks (–24 pc).[19]

Most cases of childhood acute lymphoblastic leukaemia (75 pc) were of L2 morphology which is higher than 10–15 pc in European populations.[16] Other poor prognostic features included the majority being PAS negative, 13/44 (30 pc) being T-ALL, and most presenting with a high WBC. The mean age of T-ALL was 10 years (SD = 11,6). The age peak for Common-ALL is also older than in Europeans — mean 13,3 (SD 9,0) years. Common-ALL is reported to be rare in Africa compared to T-ALL.[18,19,20] However, we found 20/66 (30 pc) typed as common ALL (all ages) and 20 pc of paediatric cases.

Eighty per cent of Common-ALL were PAS positive so this remains a good marker in the absence of more sophisticated techniques. The incidence of Common-ALL is still less than in European children (–80 pc) and adults (–50 pc).[16] No cases of L3 ALL were found in this study period though 5 cases of secondary marrow involvement by Burkitt's lymphoma were seen.

The null-ALL cases are probably more accurately called "early B-precursor ALL", but this needs to be confirmed by immunoglobulin gene rearrangement studies.

It is possible that the improving socioeconomic position of black Zimbabweans is now associated with an increasing incidence of common ALL as noted in other countries.[2,21] Continued long term studies including karyotyping are needed to confirm our findings. There is also an overall increase in the incidence of acute leukaemia in Zimbabwe which is relevant to the allocation of scarce resources — the results of treatment will be reported separately.

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