# CHROMOSOMAL ABNORMALITIES IN CHILDHOOD ACUTE LEUKAEMIA AT KENYATTA NATIONAL HOSPITAL, NAIROBI

A dissertation presented in part fulfilment for the degree of Master of Medicine (Pathology) of the University of Nairobi.

by

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March, 1989.

## DECLARATION

I certify that this is my original work and has not been presented for a degree in any other University.

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This dissertation has been submitted for examination with my approval as University supervisor.

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## LIST OF ABBREVIATIONS

KNH: Kenyatta National Hospital

del: Deletion

mar: Marker chromosome

CGL: Chronic granulocytic leukaemia

$Ph^1$: Philadelphia chromosome

AL : Acute leukaemia

ANLL: Acute non-lymphocytic leukaemia

ALL: Acute lymphocytic leukaemia

AML: Acute myeloid leukaemia

APML: Acute promyelocytic leukaemia

AMOL: Acute monocytic leukaemia

AMMOL: Acute myelomonocytic leukaemia

EL: Erythroleukaemia

FAB: French American British

Hb: Haemoglobin

PCV: Packed cell volume

MCH: Mean corpuscular haemoglobin

MCHC: Mean corpuscular haemoglobin concentration

WBC : White blood cell

MGG: May Grünwald-Giemsa stain

SBB: Sudan Black B stain

PAS: Periodic Acid-Schiff reaction

CALL: Common antigen ALL

BM: Bone marrow

g/dl: Grammes per decilitre

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## SUMMARY

This is a prospective study of 14 patients aged from ten months to 13 years admitted to KNH with acute leukaemia (AL). Chromosomal analysis was successfully performed in nine children. The clinical and laboratory features of the patients were also analysed in relation to cytogenetic abnormalities identified.

The study revealed that eight out of 14 (57%) of the patients had acute non-lymphocytic leukaemia (ANLL) and 43% had acute lymphoblastic leukaemia (ALL). Out of the five children with ANLL who had chromosomal analysis performed three (60%) had karyotype abnormalities: two patients had hyperdiploidy, one with AML $M5^a$ had 47 chromosomes, and the other with AML $M_2$ had trisomy 21; the third patient had monosomy 7. None of the four patients with ALL who had chromosomal analysis performed had any karyotype abnormalities.

Four out of the nine patients, whose karyotypes were determined, died. All the four had normal karyotypes. Two died of haemorrhage having had platelet counts less than 20 x $10^9$/l which is a poor prognostic feature. The other two died of overwhelming infections.

Surface marker studies done during the course of the study were helpful in making a final diagnosis and complemented the morphological diagnosis.

Evidence from the study shows that karyotypic abnormalities in ANLL occur as frequently as those found in other studies. The prognostic significance of the chromosomal abnormalities has not been clearly demonstrated in this study in view of the sample size. It is therefore recommended that further similar but larger studies be performed. A larger study should also bring out any associations between certain karyotypes and morphological types of acute leukaemia.

# INTRODUCTION

The normal human being has forty six chromosomes arranged in pairs (diploid, 2n) in most of the cells. The gonads normally have twenty-three chromosomes (haploid, n) and some tissues such as the liver contain cells that have multiples of the diploid number (polyploid, Xn). Numerical deviations from the normal haploid, diploid or polyploid number is called aneuploidy.

In acute leukaemia, numerical abnormalities include aneuploidy with hypodiploid (less than 2n chromosomes) and hyperdiploid (more than 2n chromosomes) cell lines. This may occur due to uneven cell division during mitosis. Structural abnormalities include deletions (del), marker chromosomes (atypical chromosomes found in sufficient metaphases to constitute an abnormal cell population or clone); pseudodiploidy (metaphases with the diploid, 2n, number but one or more atypical chromosomes or additional and missing chromosomes and balanced translocations); chromosome deletions or breaks of chromatids, and increased secondary constrictions amongst other abnormalities.

Cytogenetics is the science combining the methods and findings of cytology (the study of cells) and genetics, (the study of transmission of biological information from one generation of cells or organisms to the next). Cytogenetic analysis using banding techniques has been increasingly

applied to the study of chromosome disorders in malignant diseases in recent years.

The first findings of a chromosomal abnormality in cells from a patient with leukaemia was reported by Ford et al (1) in 1958. This prompted many others to do further research into the subject among them being Nowell and Hungerford (2, 3) who in 1960 found a characteristic minute chromosome in chronic granulocytic leukaemia (CGL). This chromosome was called the Philadelphia chromosome ($Ph^1$) after the city in which it was first described (4). It consists of a translocation of part of the long arm of chromosome 22 to the long arm of chromosome 9, t(9q+; 22q-). Many studies have been done on the $Ph^1$ chromosome and its value in the diagnosis of CGL was well documented by Kemp et al (5) as early as 1964.

Chromosomal abnormalities in AL have been found in all cytological types of AL and at all ages from the newborn to the aged (6, 7). An increase in random aneuploidy suggests a diagnosis of AL (8). Kiossoglou et al (9) in 1963 described aneuploidy in over 60% of the cases of AL they studied. Their findings were supported more recently at the First International Workshop on Chromosomes in Leukaemia (10) that was held in Helsinki, Finland in 1977.

The exact cause of AL remains obscure. A variety of possible predisposing factors have been examined including environmental factors (11-14), genetic factors (12-21), immunological factors (22-24), chemical substances (25-27)

and viruses (23, 28, 29). Several of these factors have also been shown to be associated with chromosomal abnormalities (12, 19, 24, 27, 28,). Although chromosomal changes in leukaemic cells may have a role in disease development, their precise role as initiators or promoters of the leukaemic process remains uncertain.

Using recently developed sophisticated chromosomal staining techniques and culture methods Kaneko et al (30) in 1982 were able to detect chromosomal abnormalities in over 75% of the children with ANLL. The Third International Workshop on Chromosomes in Leukaemia (31) held in 1982 found that approximately two thirds of the patients with ALL have demonstrable karyotypic abnormalities. The frequency of chromosomal abnormalities seen in patients with ANLL is different for any subtype of leukaemia according to the French American British (FAB) classification. For instance the 15:17 translocation is very high in acute promyelocytic leukaemia (AML, FAB class $M_3$) as has been described by several workers (32, 33, 34). Abnormalities of the long arm of chromosome 11 (11q) have been noted to be higher than expected in acute monocytic leukaemia (AMOL) and the association is particularly strong in children (35). The close correlation between t(18; 21) and the $M_2$ subtype of acute myeloid leukaemia (AML) is well documented (36, 37).

In ALL the karyotypic abnormalities that predominate are pseudodiploidy and hyperdiploidy. Others commonly found are translocations involving chromosomes 8 and, or

14t(14q+; 8q-), and 4 and 11t(11q+; 4q-). Chromosome 6 and 14 abnormalities are also common t(14q+; $6q^-$), (31). Virtually all cases of B cell ALL have the (14q+: $8q^-$) abnormality which appears to be specific to it. The (11, 14) ($p^{13}$, $q^{13}$) abnormality seems to be confined to T cell ALL. Nearly a third of the cases of pre-B cell ALL have demonstrated a t(1: 19) q(23: 33) abnormality (38).

The karyotype has been found to have a bearing on the prognosis in childhood AL. Williams et al (39) in 1982 demonstrated that chromosome number was the strongest single predictor of the disease outcome in children with ALL. Children with hyperdiploid (>50 category) having the best prognosis while pseudodiploid had the poorest prognosis. Those with normal karyotype and hyperdiploid (47-50 category) and hypodiploid had an intermediate response. Other studies done show that patients with the $Ph^1$ chromosome, a 14q+, t(8; 14) and t(4; 11) have the lowest response rates (31, 40, 41).

In AML several studies (10, 42) have shown that patients with a completely normal bone marrow karyotype at diagnosis have a better prognosis than those with only abnormal cells. No similar correlation has been found between the karyotypic pattern of leukaemic cells and survival in AMOL and acute myelomonocytic leukaemia (AMMOL) (10).

## JUSTIFICATION

In Africa as a whole little work has been done on childhood AL to determine the pattern of chromosomal abnormalities that occur. Bernstein et al (43) did some work on 26 South African children with ANLL where they detected chromosomal abnormalities in more than 75% of the children. As no studies on chromosomal abnormalities in acute childhood leukaemias in Kenya have been carried out the author was prompted to undertake the study to establish whether cytogenetic studies would be useful in the clinical diagnosis of the disease. A further point of interest was to find out whether the karyotypic pattern of abnormalities are similar to those found elsewhere. That this may not be the case has been suggested by the findings at the Fourth International Workshop on Chromosomes in Leukaemia held in 1982 in Chicago, Illinois, U.S.A. (36), where an increased incidence of major translocations was found in blacks and orientals as compared to whites.

## AIMS AND OBJECTIVES

**Main Objective**

The study was undertaken with the general objective of investigating the chromosomal abnormalities in childhood acute leukaemia as seen at Kenyatta National Hospital (KNH).

The specific objectives being:-

1. To determine the frequency of chromosomal abnormalities in childhood AL at KNH.

2. To determine the diagnosis of AL on morphology and by cytochemical stains then compare with that based on surface marker studies.

3. To correlate the chromosomal abnormalities with the type and morphological subtypes of AL.

4. To correlate the chromosomal abnormalities to the clinical and haematological parameters.

5. To explore, where possible, the relationship between the abnormality and the response to treatment including remission induction, remission duration and survival.

6. To compare the chromosomal abnormalities found in the study group with the those of childhood AL found elsewhere.

# MATERIALS AND METHODS

## Place and Period of Study

The study was carried out at the Kenyatta National teaching Hospital (KNH), Nairobi, Kenya in the Paediatric Oncology Ward (Ward 45), the General Paediatric wards (wards 7, 8, 9, and 10) and the Paediatric Emergency Ward (PEW). The study involved fifteen chidren and was carried out over a 9 - months period.

## Ethical Consideration

The study was carried out with the approval of the Ethical and Research Committee, K. N. H., and a written or verbal consent from the parents or guardians was granted.

## Study Population

The patients studied were those admitted to the wards mentioned above and had a diagnosis of acute leukaemia. The diagnosis was established according to the standard procedures of peripheral blood film examination, bone marrow cytology and cytochemistry utilizing the FAB classification (44).

Exclusion Criteria

(i) Patients above 15 years of age.

(ii) Patients diagnosed as having leukaemia in the past and those who had previously received treatment for leukaemia in a hospital.

Patient Workup

Patients satisfying the criteria for inclusion had a full history of their illness taken, a physical examination performed and the information entered into a study proforma sheet (see appendix I). The following physical findings that may have been related to leukaemia were emphasised in the physical examination:

- Pallor
- haemorrhagic tendencies
- fever
- infection
- lymphadenopathy
- organomegaly
- gum hypertrophy
- bone pains
- chloromas
- soft tissue swelling
- superior vena caval syndrome

Other features such as arthropathy, jaundice and features of neurological involvement were noted. Patients were treated according to laid down protocols (see appendix II) after a complete haematological workup. The results of induction therapy and remission duration and length of total survival were noted.

Laboratory Methods

(a) Peripheral blood examination.

Venous blood collected in ethylenediamine tetra acetate (EDTA) was processed through the Coulter Counter model S plus IV and the following haematological indices taken:-

- haemoglobin (Hb)
- packed cell volume (PCV)
- mean corpuscular haemoglobin concentration (MCHC)
- white blood cell (WBC) count
- red cell count
- platelet count

Peripheral blood films were stained by the May Grunwald-Giemsa (MGG) technique (see appendix III) and were examined for the differential WBC count, morphology, presence and nature of blasts, red cells and platelet morphology.

(b) Cytochemical stains

Cytochemical staining was performed on peripheral blood films. These were formally requested and performed in the

Special Haematology Section of the haematology laboratory, KNH, by qualified personnel according to standard procedures. Cytochemical stains done included Sudan Black B (SBB) using the method of Sheehan and Storey, Periodic acid-Schiff reaction (PAS) using the method of Goldberg and Barka, and Feulgen Staining, using the method of Gardikas and Israels (45).

(c) Bone marrow examination.

Bone marrow was aspirated from the iliac crest by the author using the standard procedure (see appendix IV) and processed under the direction of a qualified haematologist. The type of AL was determined according to the FAB classification (44), on morphological and cytochemical grounds as for the peripheral blood film.

(d) Cytogenetic methods

Chromosomal analysis was performed on material from the peripheral blood and bone marrow by a cytogeneticist experienced in leukaemia cytogenetics at the University of Helsinki's Department of Medical Genetics in Finland. Prior preparation of the specimens before posting to Finland was performed at the K.N.H. as follows:-

Preparation of the peripheral blood:

10 mls of venous blood was withdrawn into a syringe primed with 0.4 mls of preservative-free heparin and

gently mixed for about 5-10 seconds. The blood was then transferred into a sterile Universal bottle under sterile conditions.

Preparation of the bone marrow:

2 mls of bone marrow aspirated was delivered into 0.4 mls of preservative-free heparin, (specially diluted for the bone marrow specimen) and gently agitated for about 5-10 seconds.

The specimen was then transferred to a bottle containing 10 mls of sterile culture medium (RPMI). Both the peripheral blood and bone marrow specimens were packed together in a box with a formal requisition for chromosomal analysis. The relevant details about the patient were supplied on the requisition. The specimen package was then labelled, addressed and sent by courier service so as to reach its destination within 48 hours of removal from the patient.

At the Department of Medical Genetics in the University of Helsinki the nucleated cells were separated from the rest by Ficoll-Paque density-gradient centrifugation. These cells were then cultured in RPMI medium containing 20% fetal calf serum. Some cells were then used for chromosomal analysis using the G-banding technique (see appendix IV) and the rest of the cells were used for surface marker studies.

(c) Surface marker studies.

These were performed on the same blood/and bone marrow specimens as explained above, using monoclonal antibodies. The results of the cytogenetic and surface marker studies were posted to the Haematology Department of K. N. H.

Data analysis

The data collected was analysed using tables and histograms according to the parameters and variables under study. Percentages where necessary were also employed.

## RESULTS

A total of 15 patients were studied as detailed in the materials and methods. One of the patients (Number 9) initially thought to have AL was found to be suffering from a haemolytic anaemia on re-evaluation and was thus dropped from the study.

Figure 1 is a diagramatic presentation of the age and sex distribution of the patients studied. The ages ranged from ten months to 14 years. There were more males than females in the study, the male to female ratio (M:F) being 1.8:1. In the age group 0-10 years the M:F ratio was 2.3:1. There were equal numbers of male and female patients in the 10-15 year age group.

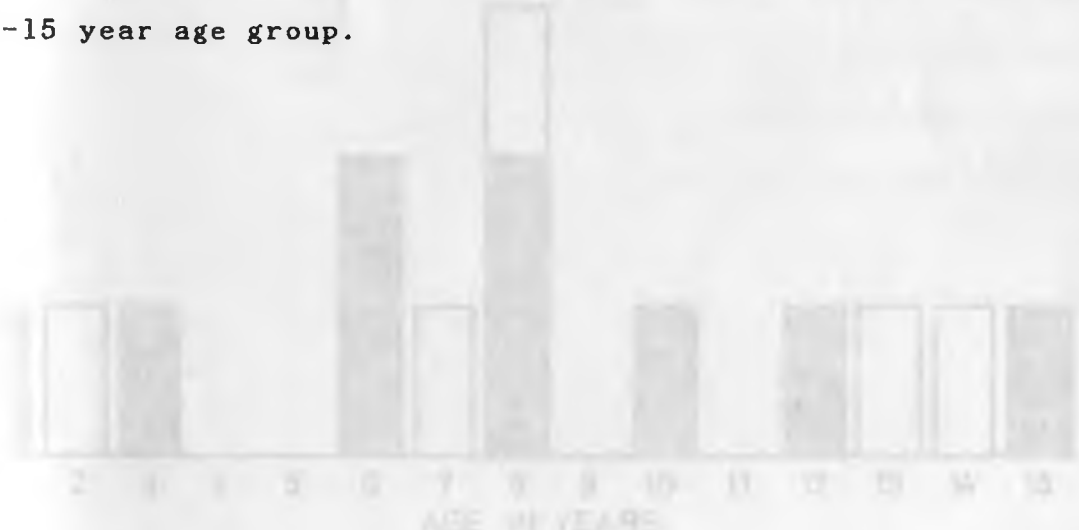

Figure 1: Histogram showing the age and sex distribution in 14 patients.

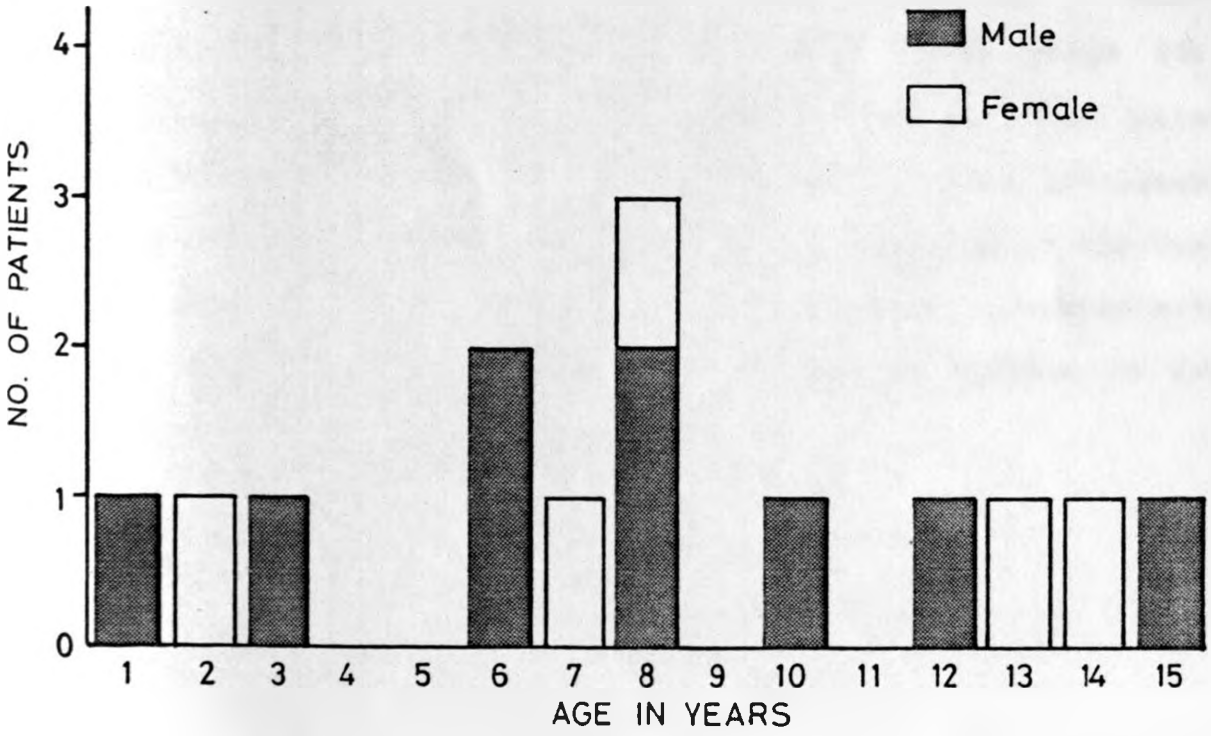

Table 1 shows the main symptomatology encountered in all the 14 study patients. In the ANLL patients (8) fever, malaise, anorexia and weight loss were the most common symptoms occurring in over 75% of the patients. A history of bone pains, bleeding gums and, or bleeding elsewhere (epistaxis and bloody diarrhoea) occurred in 50% of the patients. Approximately 38% (3) of the patients complained of cough. A history of swelling of the body or joints was given in three patients (approximately 38%) as was a history of swelling of the feet. In ALL fever was a major presenting symptom occurring in 5 out of 6 (about 83%) patients while about 67% complained of malaise. Bone pains and bleeding gums and or bleeding elsewhere were infrequent presenting symptoms (about 17%), as was swelling of the feet (about 17%). One patient (number 2) with AL presented with failure to thrive. Cough was a presenting symptom in two patients.

Table 1: The main symptoms in the study patients.

| Symptom | ANLL (n=8) | ALL (n=6) |
|---|---|---|
| Fever | 6 | 5 |
| Anorexia | 7 | 3 |
| Weight loss | 7 | 2 |
| Malaise | 6 | 4 |
| Cough | 3 | 2 |
| Vomiting | 2 | 1 |
| Bone pains | 4 | 1 |
| Bleeding gums | 2 | 0 |
| Bleeding elsewhere | 2 | 1 |
| Swelling feet | 3 | 0 |
| Swelling elsewhere | 2 | 1 |
| Failure to thrive | 0 | 1 |

Table 2 shows the age and sex correlated with diagnosis according to the FAB classification in the 14 patients. Also included are the results of the cytochemical staining reactions. Eight out of 14 patients (57%) had ANLL while the rest (43%) had ALL. For ANLL, $M_3$ and $M_5$ were more common subtypes having two patients each. There was one patient with subclasses $M_1$, $M_2$, and $M_6$ each. There were no patients with the $M_4$ subtype. One patient (number 7) had not yet had his ANLL subtype determined by the time of his death. One third of the six patients with ALL had the $L_1$ subtype while the other two thirds had the $L_2$ subtype.

Table 2: The age and sex vs FAB diagnosis with details of cytochemistry results.

| Patient Number | Age Yrs | Sex M/F | Cytochemical staining reactions SBB | PAS | Feulgen (no.nucleoli,size) | Diagnosis FAB class. |
|---|---|---|---|---|---|---|
| 1 | $2^1/_2$ | M | + | ND | ND | AML $M_6$ |
| 2 | 10 mon. | M | 0 | 1-2 granular (40%) | 1-2 small | ALL $L_1$ |
| 3 | 7 | F | 0 | 1 granular (11%) | 2-3 small | ALL $L_2$ |
| 4 | 6 | F | 0 | 0-1 granular (<5%) | 2-3 medium | AML M5[a] |
| 5 | 11 | M | 0 | 1-2 granular (20%) | 1-2 small | ALL $L_2$ |
| 6 | 5 | M | ND | ND | ND | AML $M_1$ |
| 7 | 7 | M | +/- | 0-1,diffuse (<5%) | 1-2 medium | AML $M_?$ |
| 8 | 7 | M | 0 | 1 granular (2%) | 1-2 medium | ALL $L_2$/ALSCL |
| 10 | $1^2/_3$ | F | ND | ND | ND | AML $M_5$ |
| 11 | 13 | F | 0 | 1-2,granular,(50%) | 1-2 small | ALL $L_2$ |
| 12 | 14 | M | + | 1, weak,diffuse(20%) | 1-2 medium | AML $M_2$ |
| 13 | 9 | M | + | ND | ND | AML $M_3$ |
| 14 | 12 | F | 0 | 1-2,granular,(70%) | 1-2 small | ALL $L_1$ |
| 15 | 5 | M | 0 | 2 diffuse(>50%) | 2 small | AML $M_2$ |

Key: M - Male
F - Female
SBB - Sudan Black B
PAS - Periodic acid-Schiff's reaction
parentheses after PAS indicate % blasts positive
ND - Not Done
+/- - weak (few positive cells)
0 - no reaction
1 - moderate
2 - moderate to strong
mon - months

Table 3 shows some vital statistical and clinical features that are considered to be potentially poor prognostic indicators in childhood AL (46). The data included the age and sex of the patient. Age less than 2 years and greater than 10 years is considered as a poor prognostic indicator. Belonging to the male sex is also a poor prognostic factor. Two out of the 14 (14.3%) patients were less or equal to 2 years of age, while four (28.6%) patients were greater than 10 years of age. This makes a total of 42.9% patients in the age risk group. Nine out of the 14 (64.3%) patients were males. The clinical features at presentation included the following poor prognostic indicators: fever or infection which was found in 10 (71.4%) patients, orbital chloroma in two patients (numbers 12 and 13), gingival hypertrophy in one patient (number 12). The two patients (numbers 12 and 13) with orbital chloroma had AML $M_3$. Hepatomegaly and, or splenomegaly of greater than 5 cm, was encountered in five patients (numbers 1, 2, 4, 6, and 11). Malnutrition or wasting at presentation was found in five patients (numbers 2, 6, 7, 8, and 11).

Additional findings in the patients included pallor found in all patients except one (number 3). Haemorrhagic stigmata mainly in the form of epistaxis, petechiae of gums or lips was found in six (43%) of the 14 patients. More cases of ALL (60%) had haemorrhagic stigmata as compared to ANLL (33%). One patient (number 7) with ANLL presented with bloody diarrhoea with no apparent parasitic or bacterial

cause. Patient number 8 with ALL had bilateral parotid enlargement while another (number 14) had arthropathy. Five (35.7%) of the patients had presented with oedema (periorbital or pedal or both) which was attended by low Hb values of less than 6 g/dl.

Table 3: Potentially poor prognostic indicators

| Prognostic features | Number of cases N=14 | % |
|---|---|---|
| Vital Statistics: | | |
| Age: <2 and >10 years | 6 | 42.9 |
| Sex: Male | 9 | 64.3 |
| Clinical: | | |
| Gingival hypertrophy | 1 | 7.1 |
| Spleen/Liver > 5 cms | 5 | 35.7 |
| Orbital chloroma | 2 | 14.3 |
| Malnutrition | 5 | 35.7 |
| Fever or infection | 10 | 71.4 |

Table 4 shows the fate of the specimens shipped for cytogenetic studies. Two specimens (from patient numbers 3 and 5) were lost in transit. One specimen (from patient number 12) had no viable cells by the time it reached the destination. Only eleven specimens were therefore suitable for chromosomal analysis.

Table 4: Fate of samples shipped to Helsinki.

| Patient Number | Sample sent | | State of sample | | | Comment |
|---|---|---|---|---|---|---|
| | Blood | Bone marrow | intact/ good state | unsuitable for processing | Lost in Transit | |
| 1 | Yes | Yes | Yes | - | - | Alot of bacteria in samples ? sepsis |
| 2 | Yes | Yes | Yes | - | - | - |
| 3 | Yes | Yes | - | - | Yes | |
| 4 | Yes | Yes | Yes | - | - | - |
| 5 | Yes | Yes | - | - | Yes | - |
| 6 | Yes | Yes | Yes(BM) | Yes(blood) | - | Blood was coagulated |
| 7 | Yes | Yes | Yes | - | - | - |
| 8 | Yes | Yes | Yes | - | - | - |
| 10 | Yes | Yes | Yes | - | - | - |
| 11 | Yes | No | Yes(blood) | - | - | - |
| 12 | Yes | Yes | - | Yes | - | All cells dead |
| 13 | Yes | Yes | Yes | - | - | - |
| 14 | Yes | Yes | Yes | - | - | - |
| 15 | Yes | No | Yes(blood) | - | - | - |

Key:

BM - Bone marrow

Table 5 shows the karyotype correlated with age and FAB morphological classification of AL in nine children. There were no mitotic figures on samples from patient numbers 6 and 13 hence chromosomal analysis could not be done. Of the nine patients with AL who had chromosomal analysis successfully carried out five (about 56%) had ANLL and four (about 44%) had AL. Three (about 60%) patients with ANLL had abnormal karyotypes while none of the patients with ALL had demonstrable abnormalities. There were two cases of hyperdiploidy: these were patient 4 with AMOL ($M5^a$) a female of six years who had forty seven chromosomes and an unknown extra marker chromosome; then patient 15, a male child of five years of age who had trisomy 21 (Down's syndrome). The third patient with a chromosomal abnormality was patient number 1, a two and a half year old male child with erythroleukaemia ($M6$); he had monosomy 7 and a marker chromosome which was very tiny and may have contained the centromeric area of the other missing chromosome 7.

Table 5: Results of karyotyping correlated with age and FAB morphological classification.

| Patient Number | Karyotype | Age (Yrs) | Type of Leukaemia (FAB class) |
|---|---|---|---|
| | | | **ANLL** |
| 15 | 47 XY + 21 (B) | 5 | AML $M_2$ |
| 4 | 47 XX (B) | 6 | AMOL $M_5$ |
| 10 | 46 XX (B,BM) | $1^2/_3$ | AMOL $M_5$ |
| 1 | 46 XY,-7+mar(B) | $2^1/_2$ | EL |
| 7 | 46 XY (B) | 7 | undetermined |
| | | | **ALL** |
| 2 | 46 XY (B) | 10 months | $L_1$ |
| 14 | 46 XX (B) | 12 | $L_1$ |
| 8 | 46 XY (B) | 7 | $L_2$ |
| 9 | 46 XX (B) | 13 | $L_2$ |

Source of mitotic cells:-

B - Blood

BM - Bone marrow

Table 6 shows some clinical and haematological data and the karyotype results. The remission induction results in patients who received chemotherapy are on the same table. The haematological parameters were those found at presentation. All the patients presented with anaemia with Hb values ranging from 2.8 g/dl to 10.7 g/dl. One of the patients (number 2) with an Hb of 10.7 g/dl had been previously transfused and this had therefore raised the Hb level. About half the patients had WBC counts higher than normal ranging from 31 x $10^9$/l to 91 x $10^9$/l. The patients with ANLL tended to have higher WBC counts than those with ALL, while three (75%) patients with ALL had less than 10,000 x $10^9$/l. The lowest WBC count was 2.9 x $10^9$/l found in a patient (number 2) with ALL-$L^1$ while the highest count 91 x $10^9$/l occurred in a patient (number 4) with AML-$M5^a$. Of the 14 patients, 44% had platelet counts of over 100 x $10^9$/l while 56% had counts lower than 50 x $10^9$/l. Hepatomegaly was a common feature and in only two patients (numbers 10 and 15), both with ANLL was the liver not enlarged. Splenomegaly was a less common feature and was found in five of the nine patients. All the patients without splenomegaly except for one (number 8) had ANLL. Lymphadenopathy was encountered in 67% of the patients, both with ANLL and ALL. All the three patients with abnormal karyotypes had low Hb values ranging between 4 g/dl to 6.4 g/dl. Two of these patients also had high WBC counts of 91 x $10^9$ and 35 x $10^9$/l respectively.

Table 6: Clinical and haematological data vs karyotype results

| Patient Number | Diagnosis | Age(Yrs) Sex(M/F) | Karyotype | Hb g/dl | WBC x10⁹/l | Platelets x10⁹/l | LD | H | S | Response to therapy |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | AML ($M_6$) | $2^1/_2$ M | 46 XY-7+mar | 4 | 35 | <50 | + | + | + | CR⁺ |
| 2 | ALL ($L_1$) | $^5/_6$ M | 46 XY | 10.7 | 2.9 | <50 | + | + | + | Died |
| 4 | AML ($M5^a$) | 6 F | 47 XX | 4 | 91 | 120 | - | + | + | PR⁺ |
| 7 | AML (M?) | 7 M | 46 XY | 2.80 | 18.5 | <10 | + | + | - | Died |
| 8 | ALL($L_2$/ALSCL) | 7 M | 46 XY | 9.40 | 6.6 | 350 | + | + | - | IF |
| 10 | AML ($M_5$) | $1^2/_3$ F | 46 XX | 9.60 | 54.1 | 20 | + | - | - | Died |
| 11 | ALL ($L_2$) | 13 F | 46 XX | 5.40 | 7.1 | 140 | + | + | + | CR⁺ |
| 14 | ALL ($L_1$) | 12 F | 46 XX | 7.30 | 87.3 | 50 | - | + | + | CR⁺ |
| 15 | AML ($M_2$) | 5 M | 47 XY+21 | 6.40 | 8.4 | >100 | - | - | - | PR⁺ |

Key:

M - Male F - Female +mar - marker chromosome
LD - Lymphadenopathy H - Hepatomegaly
S - Splenomegaly IF - Induction failure
CR - Complete remission PR - Partial remission

a '+' after CR, or PR indicates patient still in remission

Table 7 details results of karyotype in relation to FAB classification, remission status and duration of survival after admission. Three (60%) out of five patients with ANLL and two (50%) out of four patients with ALL are alive at the time of reporting this study. Patient 1 with EL ($M_6$) achieved bone marrow (BM) remission after five courses of chemotherapy, three months after initiation of therapy. At the time of reporting this study he is on maintenance therapy and attending the Haematology clinic for follow-up. His survival so far is seven months. Patient 14, also with ALL-$L_1$ also achieved complete remission about one month after starting remission induction; so far her total survival is three months. Patient 11 achieved complete BM remission after about two and a half months therapy. The two patients with AML and hyperdiploidy (patient numbers 4 and 15) have achieved partial remission. Patient 4 so far has had a total survival of five months while patient 15, three months. Both are still undergoing cytoreduction at the time of writing.

Of the four patients who died, three (numbers 2, 7, and 10) died prior to the onset of chemotherapy. The other patient (number 8) had induction failure (IF). All four patients had a survival of about half a month each. Of those who died, half of them were less than two years old. Half of them had AML ($M_5$ and undetermined subtypes respectively), while the other half had ALL. All the four

patients who died did not have any demonstrable karyotypic abnormalities.

Table 7: FAB classification and karyotype correlated with remission status

| Patient Number | FAB Class | Age (Yrs) | Karyotype | Survival (months) |
|---|---|---|---|---|
| 1 | RL ($M_6$) | $5^1/_2$ | 46 XY -7+mar | 7 |
| 2 | ALL ($L_1$) | $^5/_{12}$ | 46 XY | 0.5 Died |
| 4 | AMOL (M5*) | 6 | 47 XX | 5 |
| 7 | ASLL (UD) | 7 | 46 XY | 0.5 Died |
| 8 | ALL ($L_2$) | 7 | 46 XY | 0.5 Died |
| 10 | AMOL ($M_6$) | $1^8/_{12}$ | 46 XY | 0.5 Died |
| 11 | ALL ($L_2$) | 13 | 46 XX | 4 |
| 14 | ALL ($L_2$) | 12 | 45 XY | 3 |
| 15 | AML ($M_2$) | 5 | 47 XY + 21 | 3 |

Key: mar - marker
UD - undetermined

Table 7: FAB classification and karyotype correlated with with remission status

| Patient Number | FAB Class | Age (Yrs) | Karyotype | Survival (months) |
|---|---|---|---|---|
| 1 | EL ($M_6$) | $2^1/_2$ | 46 XY -7+mar | 7 |
| 2 | ALL ($L_1$) | $^5/_6$ | 46 XY | 0.5 Died |
| 4 | AMOL ($M5^a$) | 6 | 47 XX | 5 |
| 7 | ANLL (UD) | 7 | 46 XY | 0.5 Died |
| 8 | ALL ($L_2$) | 7 | 46 XY | 0.5 Died |
| 10 | AMOL ($M_5$) | $1^2/_3$ | 46 XY | 0.5 Died |
| 11 | ALL ($L_2$) | 13 | 46 XX | 4 |
| 14 | ALL ($L_2$) | 12 | 46 XY | 3 |
| 15 | AML ($M_2$) | 5 | 47 XY + 21 | 3 |

Key: mar - marker
UD - undetermined

Table 8 outlines the causes of death in four patients. Included are some of the data thought to play a role in survival of patients such as karyotype age, morphological type of leukaemia (FAB classification), initial WBC and platelet counts. A postmortem examination was performed on patient 7. Two of the four patients (numbers 7 and 10) both with ANLL died from intratranial haemorrhages. Patient number 7, in addition, had bled into the gastrointestinal and genitourinary tracts and lungs. Both these patients had very low platelet counts of 10 x $10^9$/l (number 7) and 20 x $10^9$ (number 10). The other two patients who died (numbers 2 and 8) had ALL and died of overwhelming infections. Their white cell counts were 2.9 x $10^9$/l and 6.6 x $10^9$/l respectively.

Table 8: Causes of death in four patients in relation to diagnosis, karyotype, and haematological parameters.

| Patient Number | Diagnosis | Karyo-type | Hb g/dl | WBC x$10^9$/l | Platelet count x$10^9$/l | Cause of Death |
|---|---|---|---|---|---|---|
| 2 | ALL ($L_1$) | 46 XY | 10.7 | 2.9 | < 50 | overwhelming infection |
| 8 | ALL ($L_2$) | 46 XY | 9.4 | 6.6 | 350 | overwhelming infection ? septicaemia |
| 10 | AMOL | 46 XX | 9.6 | 54.4 | 20 | intracranial haemorrhage |
| 7* | ANLL | 46 XY | 2.8 | 18.5 | < 10 | severe haemorrhage (intracranial,GIT,GU, lungs) |

Key:

* - Postmortem performed

GIT - Gastrointestinal tract

GU - Genitourinary tract

Table 9 shows the comparison of initial diagnosis based upon the clinicopathological presentation, morphology and special staining, and the final diagnosis based upon surface marker studies done in the course of the study. This comparison was not possible in patients who did not have surface marker studies as was the case in patient 1 whose samples were bacteriologically contaminated and patient 12 who did not have viable cells in the samples on arrival at the destination. The initial and final diagnoses were compatible in all cases except one: patient 6 had an initial diagnosis of AML-$M_1$, however marker studies revealed that more than 90% of the bone marrow cells were positive for glycophorin A an erythroid marker, thus suggesting that the leukaemia was of erythroid lineage. In patient 13 with an initial diagnosis of AML-$M_3$, the marker studies suggested a double lineage AL. However in patients 14 and 15, the cell lineages for their leukaemia were not confirmed because the surface markers used were not reactive with their cells. In case of patient number 14, the cells were non-reactive with MY7, (a granulocyte marker), J5 (CALL), glycophorin A (erythrocyte) and Leu 14 (B cells). This suggests that the leukaemia may have been of T cell origin or was a null ALL. In patient number 15 with an initial diagnosis of AML $M_2$ his leukaemic cells were non-reactive for the two surface markers studied (MY7 and J5). Further surface marker studies may have helped elucidate the cell lineage of the leukaemia.

Table 9: Comparison of cytomorphological diagnosis and diagnosis based on surface markers.

| Patient Number | Initial Diagnosis | Surface marker studies | Final diagnosis | Comparison |
|---|---|---|---|---|
| 1 | AML ($M_6$) | ND-samples had alot of bacteria | - | - |
| 2 | ALL ($L_1$) | Blasts J5$^+$ (weakly positive) | ALL | Compatible |
| 4 | AML (M5$^a$) | MY7$^+$, J5$^-$ | AML | Compatible |
| 6 | AML ($M_1$) | >90% BM cells GPA + | AML-?$M_6$ | Not compatible |
| 7 | ANLL | Blasts 16k 7++, MY 7 +/- | AML | Compatible |
| 8 | ALL ($L_2$ ALSCL) | Blasts J5 + | ALL | Compatible |
| 10 | AML ($M_5$) | Blasts MY4 +, MY7 +/- | AML | Compatible |
| 11 | ALL ($L_2$) | J5 + | ALL | Compatible |
| 12 | AML ($M_3$) | ND - cells unsuitable | - | - |
| 13 | AML ($M_3$) | MY 7 +, J5 + | Double lineage AL | |
| 14 | ALL ($L_1$) | Blasts MY7 -, J5-,GPA-,leu 14- | T cell or null ALL | |
| 15 | AML ($M_2$) | Blasts MY7-,J5- | Unclassified AL | |

Key:

J5 (CD10) - for common antigen ALL
MY7 (CD12)- for granulocytes
MY4 (CD14)- for granulocytes/monocytes
16k7(CD unknown)-for eosinophils
Leu 14 (CD23) - for B cells
ND - Not determined
GPA- Glycophorin A for erythrocytes

## DISCUSSION

Data from this study have revealed cytogenetic abnormalities occurring in three out of five (60%) patients with ANLL. This figure is comparable to the frequency of 60 - 75% found in the studies done elsewhere (9, 30, 43). Two of these studies (30, 43) used sophisticated high resolution chromosomal banding techniques and were thus able to detect chromosomal abnormalities in 75% of their patients. One would have expected a higher rate of abnormalities in the present study to be in line with the data from the Fourth International Workshop on Chromosomes in Leukaemia (36) which indicated that Blacks had a higher incidence of cytogenetic abnormalities than Whites. Probably the use of more sophisticated techniques in a future study would detect subtle abnormalities such as translocations and minute deletions. None of the four patients with ALL exhibited any demonstrable chromosomal abnormalities by the methods used. Studies on ALL done elsewhere (31) have shown that about two thirds of children with ALL have chromosomal abnormalities of one form or another (36). However, the number of only four patients with ALL may have been too small to come across those with abnormalities.

Of the 14 patients studied eight (57%) had ANLL while the rest (43%) had ALL. This is not surprising for although ALL has been noted to occur three times more frequently than ANLL in children in the United States of America (USA) (23),

Kasili (47) in his study of acute leukaemia in Kenyan children showed that ANLL is more common than ALL in African children. Bernstein et al (43) also found that 52% of forty six black children with leukaemia had ANLL whereas only 11% of forty two white children had the disease.

The sex distribution of acute leukaemias in the present study conforms to what has been found in Kenya for Kasili (47) reported that there was a male preponderance of AL in the first decade with a ratio of 2:1, male to female, in the age groups 0-4 and 5-9 years. In this study there is a 2.3:1 male to female ratio in the 0-10 year age group. In the second decade a female preponderance was reported. The present study had only four patients in this age group with a male to female ratio of 1:1.

The patient age can be correlated to FAB class and cytogenetic findings. In this study there were two patients (numbers 4 and 6) with AML $M_5$. One of them (number 6) was an infant (less than two years old). Studies done locally (47) have indicated that most cases of AMOL occur within the first two decades of life. The Fourth International Workshop on Chromosomes in Leukaemia (36) noted an excess of AMOL among infants (less than two years old) as compared to the other FAB subtypes of acute leukaemia. Monosomy 7 was found in a two and a half year old male with erythroleukaemia (EL). Rowley et al (48) found that there was a high percentage of patients (63%) with abnormal

karyotypes in EL. Loss of chromosome number 7 and, or number 5 occurred in 30% of all EL patients. However chromosomal abnormalities in EL tended to occur in older age groups in whom upto 75% had an abnormal karyotype.

Monosomy 7 is a common cytogenetic abnormality that has been found in all subgroups of ANLL and in the myelodysplastic syndromes (35). In the Fourth International Workshop on Chromosomes in Leukaemia (49) it was associated with low leucocyte counts, for instance, 54% of 28 patients had less than 10 x $10^9$/l WBC count while 32% had 10-50 x $10^9$/l. My case study had a presenting WBC count of 35 x $10^9$/l. Low response rates to therapy were also noted in this group especially if there was an associated chromosome 5 abnormality although patients with a $5q^-$ chromosome as a sole abnormality fared better than those with that of chromosome 7. At eight months after diagnosis of his disease the patient in this series is still alive after attaining bone marrow remission three months after starting chemotherapy. This survival is somewhat better than that reported at the Fourth International Workshop (49) where low response rates and higher induction death rates ranging from 29-33% were noted as compared with most of the other chromosome groups. Uniformly low survival rates of 1.5 - 6 months were noted in 28 patients.

Bitter et al (35) in 1986 showed that abnormalities of chromosome 5 and, or 7 are present in 90% of patients with

therapy related ANLL or myelodysplastic syndromes, suggesting possible mediation by undetected toxic agents. My case study did not have any history of exposure to known toxic agents. In children, monosomy 7 is a distinctive multipotential stem cell disorder associated with repeated infection and abnormal neutrophil chemotaxis and bacterial killing. The disorder rapidly transforms to overt leukaemia. My patient presented with fever and conjunctivitis. While in hospital he developed a malarial infection due to *Plasmodium falciparum* that was successfully treated. On another occasion he developed an upper respiratory tract infection. The blood and bone marrow specimens taken soon after admission for the study had bacterial sepsis implying that he either was suffering from septicaemia or that the specimens were contaminated during their removal from his body. It would have been noteworthy to assess his neutrophil function to find out if it was abnormal.

Another patient (number 15), a five year old male child, previously diagnosed as a case of Down's syndrome (trisomy 21) presented with AML FAB $M_2$. Chromosomal analysis confirmed trisomy 21. Down's syndrome is a constitutional chromosomal abnormality associated with upto a 20-fold increased risk of leukaemia (26). Amongst patients with constitutional abnormalities with leukaemia, Down's syndrome is the most common abnormality in both lymphocytic and non-lymphocytic leukaemia accounting for 69% and 31% of patients with ALL and ANLL respectively (51). AL

in Down's syndrome occurs at a younger age peak than in the general paediatric population. Although these patients were thought to have primarily AML, Rosner and Lee (51) provided evidence that the morphological types of AL in Down's syndrome had the same distribution as seen in leukaemia children in general. Therefore in Kenya, because the incidence of ANLL is roughly equal to that of ALL (47), it is expected that the incidence of ANLL in Down's syndrome would be about equal to that of AL. This is different from what has been noted elsewhere (51, 52) where ALL in Down's syndrome occurs more than twice as frequently as ANLL.

Kaneko and his associates (53) in their study of chromosomal abnormalities in Down's syndrome suggested that most children with Down's syndrome and ANLL have hyperdiploidy usually related to gains in C, F, and, or G chromosomes. Abnormalities of +8, +19, +22 in Down's syndrome may be associated with AL in the early stages of myeloid differentiation.

Another patient (number 7), a seven year old male child with a diagnosis of ANLL (FAB class undermined prior to his death), had in his bone marrow a preponderance of eosinophils and eosinophil precursors. His karyotype (determined from the peripheral blood) was 46 XY and surface marker studies were heavily positive for 16k7 - an eosinophil marker. MY7, the granulocyte marker was equivocal. Bone marrow eosinophilia in ANLL has been

previously described by Arthur and Bloomfield (54) in 1983 and they reported a strong association with abnormality of chromosome 16,del(16)(q 22). Three fifths of the patients with this abnormality were classified as having AML $M_2$ subtype, while the rest had the $M_4$ subtype.

The patients whose karyotypes were determined and later died, all, except one (patient 8 who had induction failure), died prior to initiation of cytotoxic therapy. These patients presented late in the course of their illness and either had severe anaemia, as exemplified by patient 7 with an Hb of 2.8 g/dl, or thrombocytopenia as in patients 7 and 10. Their low platelet counts of less than 20 x $10^9$/l were directly implicated in the causation of death and are known to be poor prognostic features (46). Another patient (number 2) presented with severe bronchopneumonia that did not resolve despite intensive chemotherapy indicating compromised immunocompetence. The significance of the karyotype in these fatal cases has not been brought out by the study unlike what has been reported in the literature (31, 39, 40, 41). It may perhaps be stipulated that subtle karyotypic abnormalities have not been detected in the present study or the number of patients was not large enough. This is an indication for further studies in order to explore the possible relationships between karyotype and survival. The leucocyte count is generally regarded as a strong predictor of treatment outcome (39) and the use of

this factor and chromosome number in AL in concert may permit a sharper delineation of prognostic groups.

Four cases of ALL had their surface marker status determined. Three (75%) of patients (numbers 2, 8, and 11) were positive for CALL on their blasts. This figure is higher than expected as studies done locally by Kasili and Dearden (55) (unpublished material) indicated that only 20% (of 20 children) had positive markers for common ALL subtype in our local population. Reports from other regions (56) indicate, however, that 74% of childhood ALL are CALL positive. More data needs to be obtained from further studies to investigate these apparent disparities.

Surface marker studies proved very useful in case of patient 6 (initial diagnosis AML-$M_1$) whose final diagnosis was amended to AML-$M_6$ after the majority of the bone marrow cells were found to be reactive for glycophorin A, a major membrane sialoglycoprotein. It is exclusively found on cells of the erythroid lineage and expressed during erythroid differentiation. In case of patient number 13, whose initial diagnosis was AML $M_3$, suface marker studies were helpful in making a final diagnosis as they indicated that this was a double lineage AL. However when one is considering the usefulness of the surface markers it should be born in mind that they play a limited role in that there is considerable overlap between some of the markers. For example, MY7 for granulocytes and MY4 for

granulocytes/monocytes will be positive in cases of $M_4$ or $M_5$ subtypes of of AML. Cytomorphological diagnosis will thus remain the preferred method, to be complemented by surface markers, especially in the not-so-clear cut cases. Surface marker studies undeniably play a role especially in ALL for predicting the prognosis. In this study two patients with ALL are alive at the time of reporting the study. One of the patients (number 11) was positive for CALL, the other (number 14) was negative.

## CONCLUSIONS AND SPECULATIONS

1. The frequency of chromosomal abnormalities in ANLL found from this study is similar to that found elsewhere. However, for ALL, further studies with larger series of patients are needed to establish whether the frequency is also as high as reported in the literature.

2. Black children have a higher frequency of chromosomal abnormalities in ANLL than white children.

3. Some types of chromosomal abnormalities may correlate to the FAB class of AL in case of ANLL.

4. There may be a relationship between karyotypic abnormalities and environmental mutagens.

5. A platelet count of less than 20 x $10^9$/l is a poor prognostic feature.

6. Surface markers are useful in making a final diagnosis on the type of AL although a diagnosis based on morphology is essential.

## CONSTRAINTS

Several problems and constraints were encountered while performing this study. The major problems were those arising because the samples were sent abroad for chromosomal analysis and surface marker studies. This meant that the samples had to be taken as early as possible during the daytime, packaged and then taken to the courier service. Samples could only be sent on Mondays to Thursdays so that the specimens would arive at the destination on or before Saturday. The weather in Finland appears to have affected one of the specimens (number 12) because it arrived during the snowing months and thus may have frozen because there were no viable cells left. This problem may be solved by insulation of the specimens during packaging if they are to be sent during the winter season. The loss of specimens in transit was a serious problem. Better streamlining of the transfer of the specimens from the hospital to the courier service point and from the courier to find the destination may help solve the problem. If the specimens were to arrive during the weekend then the recipient (cytogeneticist) would have to have prior knowledge.

Another major problem was of a financial nature as sending the specimens by courier services proved to be very expensive. An extended study would need greater funding or grants that would also cover costs of surface marker studies as monoclonal antibodies are also very expensive.

A constraint that was initially encountered in the beginning of the study but was later overcome was mainly logistical. Many of the patients presenting at PEW initially had obscure diagnoses made such as, pyrexia of unknown origin (PUO), for example. Other diagnoses like arthritis did not point out to the true nature of the disease. These diagnoses would be written down in the admission books to confuse the study issue as only after investigations were the patients shown to be leukaemic. The original diagnoses of PUO or arthritis for example, were rarely changed and thus the author would only get to know of these patients at a later date. In many cases the patient had transfered to the General Paediatric wards and treatment already initiated. The problem was later overcome after doctors attending the patients in PEW were made more aware of the ongoing study.

Bacterial contamination of specimens from patient number 1 was another problem. It was however, difficult to rule out a septicaemia in the patient. If the bacterial origin was exogenous good aseptic techniques would have to be employed to prevent this kind of problem.

One last major constraint in this study was the paucity of mitotic cells in the bone marrow specimens. Out of nine bone marrow specimens that reached the destination in a good state only one had mitotic figures in it. Perhaps a larger bone marrow aspirate may yield mitotic figures.

## RECOMMENDATIONS AND FUTURE PERSPECTIVES

This study has served as a pilot study in determining whether cytogenetic analysis performed on our leukaemic patients would be worthwhile. Despite the constraints encountered in the course of the study, the costs have been justified because the results are beneficial not just to the scientific community, but also to the patient in the diagnosis and management of his disease. It is with this in mind that the following recommendations have been made in order to pave the way for future progress.

1. That a larger study involving more patients be carried out so that the patients can be followed up for longer periods of time.

2. There is a need to explore the possible relationship between karyotype abnormalities in ANLL and potential environmental mutagens such as insecticides and agrochemicals that are widely used here. An ongoing study on aplastic anaemia in the Department of Haematology at KNH indicates that there is a correlation between it and the use of agrochemicals in this country, (unpublished material).

3. Patients with AL who are not responsive to therapy should have their karyotype determined in order to identify the poor prognostic cytogenetic groups.

4. New approaches to treating patients in the poor prognostic cytogenetic groups of AL should be explored with a view to improving their survival chances.

5. There is a demand to set up facilities here for cytogenetic studies as it is cumbersome and difficult, not to mention more expensive, to carry out a study of this nature at another centre abroad.

**ACKNOWLEDGEMENTS**

This dissertation would not have been possible without the assistance and guidance of many people, regretably not all can be mentioned.

I am indebted to my supervisor Professor E. G. Kasili of Haematology in the department of Pathology, University of Nairobi, for his invaluable assistance and guidance at the various stages of preparation of this work.

Special thanks go to: Dr. Sakari Knuutila of the Department of Medical Genetics, University of Helsinki, Finland, for performing the chromosomal analysis and surface marker studies; Farmtalia Carlo Erba, Nairobi, for undertaking the costs of specimen postage.

My appreciation and thanks are extended to my professional colleagues in Haematology for examining the bone marrow preparations; and to the paediatric ward staff for their assistance and co-operation. I gratefully acknowledge Professor R. D. Barr of McMaster University, Canada, for reading through the manuscript and offering useful suggestions.

I am also grateful for the kind assistance of Mrs. Kimani, Mr. Mugo and Mr. Mohammed of Immunology Department for assisting in the packaging of the specimens, and Mr. Ouma of Kenya Medical Research Institute for the neat word processing work.

Lastly but not least, I thank my husband for his patience and assistance in making helpful suggestions, and my children for their understanding.

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## APPENDIX I

## TREATMENT PROTOCOL FOR CHILDHOOD ACUTE LEUKAEMIA AT KENYATTA NATIONAL HOSPITAL

The standard practice of induction, cytoreduction and maintenance phases will be followed.

**Protocol: AL KNH/4 for Acute Lymphoblastic Leukaemia (ALL) (L1-L3)**

(i) **Induction:**

- Vincristine (VCR) - 2mg/m² i.v. weekly x 4
- Prednisone - 40 mg/m² p.o. in 3 doses daily, tailing off in week 6.

If remission is not attained a further dose of VCR is given or cytoreduction phase is started.

(ii) **Cytoreduction:**

Two courses starting one week after last injection of vincristine. Adriamycin is omitted during second course.

- Adriamycin 30 mg/m² i.v. (day 1-3)
- Cyclophosphamide 1200 mg/m² i.v. (saline infusion) on day 1.
- Cytosine arabinoside - 100 mg/m² i.v. O.D. (i.v. push) on day 1-5. (or methotrexate 20 mg/m² O.D. on day 1-5).

(iii) **Maintenance:**

To start after 1 week's rest and continue for 24 months

- 6 Mercaptopurine - 75 mg/m² p.o. daily
- Methotrexate - 15 mg/m² p.o. weekly
- Vincristine - 1 mg IV monthly
- Prednisone - 40 mg/m² p.o. in 3 doses daily x 7 monthly
- Adriamycin - 40 mg/m² IV every three months
- Cylophosphamide 400mg/m² IV every 3 months

**Protocol: AL KNH/4 - DMT for Acute Non-lymphocytic Leukaemia (ANLL)**

(i) **Induction/Cytoreduction:**

- Daunorubicin - 40mg/m² I.V. day 1-3
- Cytosine arabinoside - 100 mg/m² I.V. twice daily day 1-6.
- Thioguanine - 80 mg/m² (or 6 mercaptopurine 100 mg/m²) p.o. day 1-6.
- Cyclophosphamide - 1200 mg/m² I.V. in saline infusion day 6 only

The pulse is repeated after a rest period that is determined by the recovery of the haematological parameters, until complete remission is attained.

(ii) **Maintenance**: (for 24 months)

- Cytosine arabinoside - 100 mg/m² (max. 100 mg) monthly sub-cutaneously

- 6 mercaptopurine - 100 mg/m² (or 6-Thioguanine 80 mg/m²) p.o. daily days 1-5

**Management of Meningeal Leukaemia**

(a) Treatment if there is involvement at the time of diagnosis.

- Intrathecal methotrexate - 10 mg/m² (max. 12 mg) daily for five doses.

- After a 2 day rest intrathecal cytosine arabinoside 100 mg/m² (maximum 100 mg/m²) daily for 5 days.

(b) CNS prophylaxis, if there is no involvement at the time of diagnosis and when there is complete haematological remission at the beginning of maintenace therapy.

- Intrathecal methotrexate 10 mg/m² five doses in three weeks, (or cytosine arabinosinde 100 mg/m²)

- Cranial radiation, 2,500 rads in three weeks after.

**NOTE:**

**The protocols may be changed or modified if the drugs are not available.**

## APPENDIX II

Sample of Study Proforma Sheet

CHROMOSOMAL STUDY IN ACUTE LEUKAEMIA 1988-1989

NAME.....

AGE.....SEX.....

UNIT NO.....WARD ADMITTED.....

DATE OF ADMISSION.....

HAEMATOLOGICAL DIAGNOSIS.....

**CLINICAL HISTORY**

| **SYMPTOMS** | DURATION |
|---|---|
| FEVER..... | ..... |
| ANOREXIA..... | ..... |
| WEIGHT LOSS..... | ..... |
| COUGH..... | ..... |
| VOMITING..... | ..... |
| BONE PAINS..... | ..... |
| BLEEDING GUMS..... | ..... |
| OTHER (SPECIFY)..... | ..... |

**PHYSICAL FINDINGS**

PALLOR.....

HAEMORRHAGIC TENDENCIES.....SITE.....

WASTING.....

JAUNDICE.....

LYMPHADENOPATHY.....SITE.....

HEPATOMEGALY.....SIZE.....

SPLENOMEGALY.....SIZE.....

GUM HYPERTROPHY.....

SOFT TISSUE SWELLING.....SITE.....

CHLOROMAS.....SITE.....

ARTHROPATHY.....

CHEST SIGNS.....

CNS SIGNS.....

OTHER.....SPECIFY.....

PERIPHERAL BLOOD

**INVESTIGATIONS**

**PERIPHERAL BLOOD**

HB.....G/DL WBC.....x $10^9$/dl

DIFFERENTIAL WBC.....NEUTROPHILS.....% LYMPHOCYTES....%

MONOCYTES.....% EOSINOPHILS.....% BASOPHILS.....%

PROMYELOCYTES.....% MYELOCYTES.....% STABS.....%

BLASTS.....%

PLATELETS..... x $10^9$/dl

**BONE MARROW REPORT**

CELLULARITY.....MYELOID-ERYTHROID RATIO.....

ERYTHROPOIESIS.....

LEUCOPOIESIS.....

MEGAKARYOCYTES.....

PLASMA CELLS.....

ABNORMAL CELLS.....

IRON.....

CONCLUSION.....

**SPECIAL STAINS**

SUDAN BLACK B STAIN.....

PERIODIC ACID SCHIFF REACTION.....

ACID PHOSPHATASE REACTION.....

SERUM LYSOZYME CONCENTRATION.....

NON-SPECIFIC ESTERASE REACTION.....

FEULGEN STAINING.....

**CHROMOSOMAL STUDY REPORT**

.....

.....

**TREATMENT**

| Regimen | Period of treatment |
|---|---|
| 1..... | 1..... |
| 2..... | 2..... |
| 3..... | 3..... |
| 4..... | 4..... |
| 5..... | 5..... |

**OUTCOME OF TREATMENT**

| Response to initial induction | Duration | Length of Survival |
|---|---|---|
| Complete remission..... | ..... | ..... |
| Partial | | |

remission..... ..... .....

Death..... ..... .....

## APPENDIX III

### MAY-GRUNWALD - GIEMSA'S STAIN

1. Blood films are prepared on clean, dust and grease free glass slides.
2. Air dry the films .
3. Fix film by immersing in jar of methanol for 10-20 minutes.
4. Put slide into staining jar containing May-Grunwald's stain freshly diluted with an equal volumes of buffered water - 15 minutes.
5. Transfer slide, without washing, into jar containing Giemsa's stain freshly diluted with 9 volumes of buffered water - 10-15 minutes.
6. Wash rapidly in 3 or 4 changes of water and finally allow to stand undisturbed in water for differentiation to take place (2 - 5 minutes).
7. Stand slides upright to dry.
8. When thoroughly dry the slides may be mounted with a cover glass using a mountant.

## APPENDIX IV

**Bone Marrow Aspiration Method:**

**Instruments:**

1. Bone marrow aspiration tray containing sterile syringes hypodermic needles, gauze, cotton wool, draping cloth and gloves.

2. Local anaesthetic (lignocaine hydrochloride 1%).

3. Spirit or other cleansing agent.

4. Clean glass slides and spreader slide.

5. Container for transporting the prepared slides.

**Method:**

1. Place patient on right or left lateral decubitus position with knees drawn up halfway to chest.

2. Locate the Anterior superior iliac spine/posterior superior iliac spine/iliac crest site for biopsy.

3. Cleanse the operative field and drape.

4. Infiltrate skin and periosteum with local anaesthetic.

5. Secure selected needle to the Salah/Klima marrow aspiration instrument and insert it into the cortex of the bone. A 'give' will be felt when the marrow cavity is reached.

6. Remove cap of instrument and stylet and attach a syringe to needle. Aspirate about 2 mls.

7. Remove the needle from the patient. Dry and cover the area with collodium to seal the wound or use dry gauze and tape.

**Post Operative Care:**

The patient should lie recumbent in bed for a short while. Patients with bleeding tendencies are managed by applying pressure to aspiration site for 60 seconds and applying a pressure bandage. They also require careful observation for longer periods.

## APPENDIX V

Chromosomal banding technique (G-banding)

(Harlequin method).

1. Pretreat slide preparation (2 to 5 days old) with trypsin dissolved in 0.9% sodium cholride (NaCl) for 15 minutes.

2. Stain with Hoechst 33258 (0.5 ug/ml in Sorensen buffer, pH 6.8) for 15 minutes.

3. Rinse with distilled water

4. Expose to U. V. light (Philips TUV 30 W) at a distance of 1.5 metres for 20 minutes.

5. Stand on a wet sponge

6. Incubate at 60° C for 2 hours.

7. Rinse with distilled water

8. Stain for 10 minutes with Merck's Giemsa diluted 5% in Sorensen buffer pH 6.8.