Med & Health 2010; 5(1): 22-33

ORIGINAL ARTICLE

Minimal Residual Disease Status in Childhood Acute Lymphoblastic Leukaemias by Flow Cytometry and Their Clinical and Haematological Features

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ABSTRAK

Kehadiran sel leukemia yang minima merupakan penanda prognosis buruk pada pesakit leukemia akut. Penganalisaan tahap bebas leukaemia secara sitometri aliran dapat menganggarkan baki minima sel leukaemia (MRD) selepas rawatan kemoterapi induksi pada pesakit kanak-kanak leukemia limfoblastik akut (ALL). Pesakit yang dikatakan bebas dari leukaemia dengan analisa morfologi sum-sum tulang, di mana kadar peratus limfoblas adalah kurang 5%, berkemungkinan besar masih mempunyai sel leukemia sebanyak 10^{10}. Walau bagaimana pun, kaedah imunofenotip sitometri aliran, boleh mengesan baki sel leukemia yang lebih rendah lagi (1 dalam 10^4 sel-sel) berbanding pemeriksaan morfologi sumsum tulang, dan ia boleh digunakan sebagai kaedah bagi menentukan MRD dan meramal kemungkinan leukemia akut berulang. Kajian ini adalah untuk membandingkan ciri-ciri klinikal dan hematologi pada kanak-kanak yang mengalami ALL, dan juga untuk mengesan status MRD pesakit tersebut secara sitometri aliran. Status MRD pesakit dianalisa pada hari ke-28, minggu ke-12 dan minggu ke-20 daripada rawatan kemoterapi induksi leukemia. Jangka hayat pesakit selepas lima tahun juga dikaji. Perbandingan status MRD secara morfologi sum-sum tulang dengan sitometri aliran telah dilakukan. Kajian ini telah dijalankan terhadap tiga puluh lapan kes kanak-kanak yang mengalami prekursor B-ALL di Pusat Perubatan UKM. Tidak terdapat perkaitan yang signifikan diantara ciri-ciri demografik, klinikal dan hematologi dengan status MRD pada hari ke-28, namun terdapat perkaitan yang signifikan di antara status MRD yang positif secara sitometri aliran dengan keputusan morfologi sum-sum tulang pada minggu ke-12. Tiga kes menunjukkan status MRD positif yang berterusan sehingga minggu ke-20 dan dua daripadanya telah relaps dan meninggal dunia. Dua puluh empat pesakit masih hidup selepas lima tahun.

Kata kunci: baki minima sel leukaemia (MRD), leukemia limfoblastik akut (ALL), sitometri aliran, imunofenotip

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ABSTRACT

Residual disease in patients with acute leukaemia indicates unfavorable prognosis. The evaluation of remission using flow cytometry allows a better estimation of minimal residual disease (MRD) after induction chemotherapy in childhood acute lymphoblastic leukaemia (ALL) cases. Patients in morphological marrow remission with presence of blast cells of less than 5%, may still have up to 10^{10} leukaemic cells. However with flow cytometric analysis, lower levels of the residual leukaemic cells (1 in 10^4 cells) can be detected and it can be used as a tool to predict relapse. This study compared the presenting clinical and haematological features of children with ALL and their residual disease status determined by flow cytometry. Analysis of their MRD status following remission-induction chemotherapy were done at day-28, week-12 and week-20. The cases were also followed up to five years, to determine their survival status. Their residual disease status by flow cytometric immunophenotyping was also compared with their bone marrow findings morphologically. Thirty-eight cases of precursor B-ALL in pediatric patients from UKM Medical Centre (UKMMC) were analyzed. There was no significant correlation between demographic, clinical and haematological features with MRD status at day-28. However, there was a significant correlation between MRD status by flow cytometry and by morphological marrow examination at week-12. Three cases showed persistent MRD findings until week-20 where two of the cases relapsed and died subsequently. Twenty four patients were still alive after five years of follow up.

Key words: minimal residual disease (MRD), acute lymphoblastic leukaemia (ALL), flow cytometry, immunophenotyping

INTRODUCTION

Monitoring of residual disease (MRD) in leukaemic patients offers a means of evaluating the efficacy of the treatment given. In childhood acute lymphoblastic leukaemia (ALL), relapses are reported in at least 20% of children who are treated with contemporary programs of chemotherapy. However, monitoring MRD in childhood ALL may create the possibility of tailoring treatment according to patient's response, where a more intensive therapy could be given to patients with high levels of MRD and a less intense one to those without MRD. Various methods of detection of MRD in childhood precursor B-ALL, such as by morphology, karyotyping, in situ hybridization, immunofluorescence, cell culture, Southern blotting and polymerase chain reaction (PCR) analysis have been described. However, the evaluation of residual disease in acute leukaemia is most often carried out by morphological analysis of bone marrow aspirates with the criterion of free from residual disease (complete remission) denotes fewer than 5 % of blasts in the bone marrow cell population. Unfortunately, this method does not allow identification of low levels of the disease and patients in morphological remission may still have up to 10^{10} leukaemia cells (Campana & Pui 1995; Campana 2003).

Sensitive techniques to detect MRD may allow better estimation of the leukaemia burden, provides information on residual disease and aids in therapeutic strategies. Flow cytometric detection of

MRD is based on the identification of immunophenotypic combination markers expressed on leukaemic cells but not on normal haemopoietic cells. It is based on the identification of uncommon phenotypes or aberrant markers that are found at diagnosis. At certain points of time during and after chemotherapy, the same phenotypes or aberrant markers are analyzed and quantified. If expression of these markers is less than 1%, the patient is considered free from MRD. However, for the detection of a small number of leukaemic cells, a large number (10^5 - 10^6) of cells is needed for screening. Thus flow cytometry can be used as a tool to predict relapse of leukaemia patients particularly when subsequent analysis of marrow MRD levels shows an increasing trend.

The close relationship between the size of tumour burden and the curability of acute leukaemia is well established. In ALL, a high white blood cell count (WBC) at diagnosis is indicative of a large tumour mass and it is a poor prognostic indicator (Campana 2003, Pui & Crist 1994). Other presenting features such as age, sex, lymphadenopathy, organomegaly and the immunophenotype of blast cells are also known to have prognostic values in determination of remission induction rate, remission duration and long-term survival of patients. Other parameters such as haemoglobin and platelet count may play some role; however, myeloid associated markers have been proven to have no adverse prognostic significance in children (Pui et al. 2008). These parameters have been used to classify patients into standard or high-risk groups before appropriate chemotherapy is started. From previous studies, patients in the high-risk group that received standard risk of chemotherapy regime have a higher chance of relapse.

The aim of this study was to establish the detection of residual disease by immunophenotyping using flow cytometry in childhood ALL during their induction and continuing chemotherapy.

MATERIALS AND METHODS

This descriptive cross sectional study was conducted in children with precursor B-ALL aged between one and 12 years, diagnosed in UKM Medical Centre (UKMMC) from June 2001 to October 2003. It was carried out under a protocol approved by the Institutional Review and Ethics Committee. We obtained bone marrow samples from patients with informed consent at diagnosis, and at various time points that have been defined for standardized analysis of chemotherapy treatment based on the UKALL 97 protocol (Working Party On Leukaemia In Children, Revised November 1999). The time points of analysis were: following induction chemotherapy (day-28) and during the continuation therapy (week-12 and week-20) for MRD assessment. The specimens of the patients were processed in the Haematology Unit, Department of Pathology UKMMC. All patients were followed-up for five years to ascertain their survival status.

Bone marrow smears were made from the marrow aspirations of the patients for morphological analysis and the remaining of the marrow specimens were collected in sterile ethylenediamine tetraacetic acid (EDTA) tubes for immunophenotyping analysis. Differential counts of the patients from peripheral blood were also obtained at the same time. The marrow smears were stained for May-Grunwald-Giemsa's (MGG) and peroxidase cytochemistry. The morphological assessments of bone marrow smears were observed by two Pathologists and the percentage of lymphoblasts were recorded. Mononuclear cells from bone marrow aspirates collected in EDTA, were separated on a density gradient using lymphoprep (HISTOPAQUE-1077), immunolabelled with a panel of mono-

Table 1: Demographic data of the precursor B-ALL patients

Demography	Number of cases
Age	
< 2 years	7 (18.4%)
2 – 10 years	28 (73.7%)
> 10 years	3 (7.9%)
Sex	
Male	31 (81.0%)
Female	7 (19.0%)
Race	
Malay	30 (78.9%)
Chinese	5 (13.2%)
Indian	3 (7.9%)
Total	**38**

clonal antibodies conjugated with FITC and/or PE and analyzed by FACScan flowcytometer (Becton Dickinson) for immunophenotyping. Analyses of data were done using Cell Quest software (Becton Dickison).

Diagnosis of precursor B-ALL was made based on the presence of surface expression of CD19 and cytoplasmic expression of CD 22 with negative cytoplasmic myeloperoxidase (MPO). The acute lymphoblastic leukaemia cases were included in the study if the blast cells expressed at least one of the following combined immunomarkers:

i. CD19/CD34
ii. CD33/CD19
iii. CD13/CD19
iv. CD33/CD10
v. TdT

For MRD detection, bone marrow samples from all patients during follow-up visits to the hospital were analyzed with the same immunomarkers that were present at diagnosis. Figures 1 and 2 illustrate the examples of cases that were MRD positive and MRD negative respectively. Morphological assessments were also compared with the flow cytometry immunophenotyping results. Bone marrow samples from pediatric cases of idiopathic thrombocytopenic purpura (ITP) and non-haematological malignancy without bone marrow involvement were used as controls.

Demographic and clinical data such as age, sex, race, presence of lymphadenopathy, hepatomegaly or splenomegaly and blood profile of the patients upon diagnosis and at day-28 post induction chemotherapy, were also recorded.

RESULTS

Sample population

From June 2001 to October 2003, 49 children aged one to 12 years with precursor B-ALL were diagnosed in UKMMC. However, minimal residual disease (MRD) could only be studied in only 38 cases. Eleven children had to be excluded from the study because their bone marrow samples were not available at post chemotherapy or there were no suitable MRD markers detected at diagnosis. Table 1 shows the ranges of age, sex and race of patients in this study.

Clinical findings at diagnosis

Most of the children with precursor B-ALL presented with lymphadenopathy (76.3%), hepatomegaly (89.5%) and splenomegaly (76.3%). Some of these cases had very large liver and spleen (≥5.0 cm and below subcostal margin) i.e. 11 (28%) and 8 cases (21%) respectively.

White blood cells at diagnosis

Table 2 illustrates the haematological characteristics of the patients at diagnosis. Most of the cases presented with white blood cell counts (WBC) of less than 50.0×10^9/L. Only four cases (10.5%) had WBC more than 50.0×10^9/L with the

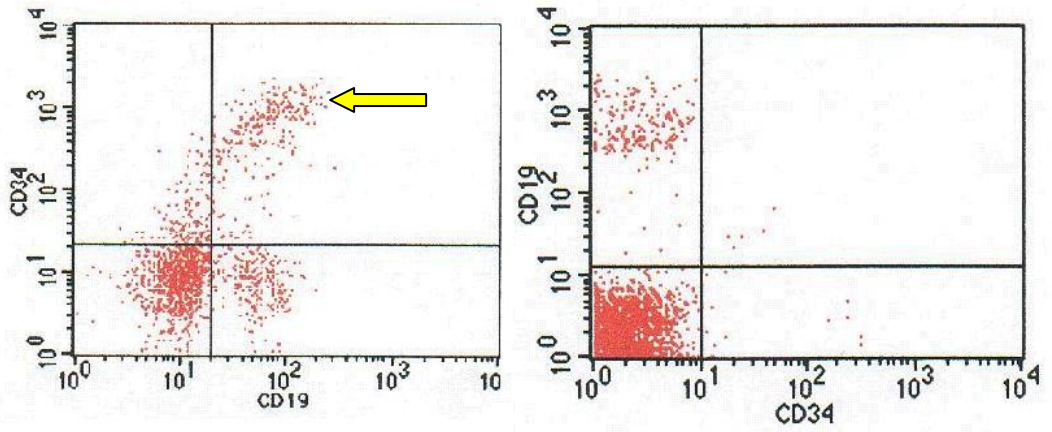

Figure 1: Flow cytometric analysis using two leukaemia-associated immunophenotypic markers showing presence of MRD (left – arrow) and absence of MRD (right).

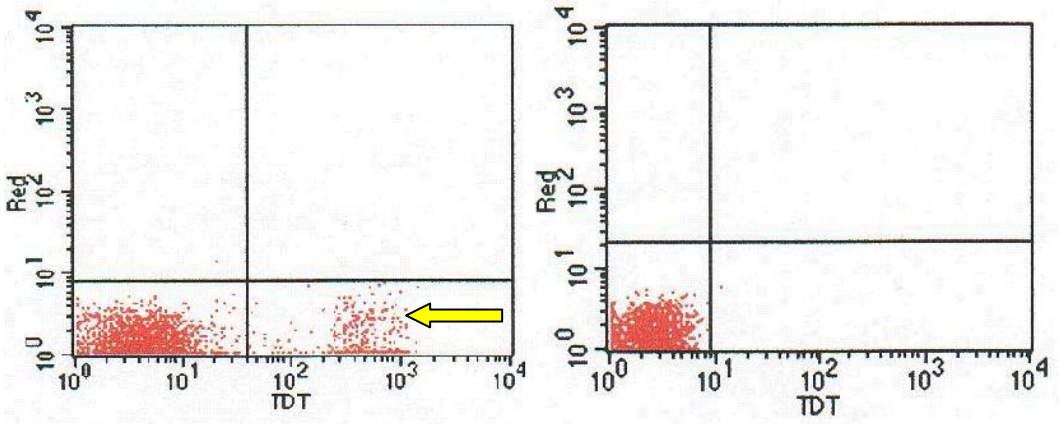

Figure 2: Flow cytometric analysis using a single leukaemia-associated immunophenotypic marker showing presence of MRD (left – arrow) and absence of MRD (right).

Table 2: Haemoglobin, white cell count, and platelet parameters of precursor B-ALL cases at diagnosis and at post remission induction chemotherapy

	Minimum	Maximum	Mean
At diagnosis			
WBC x 10(9)/L	2	322	29.07
Hb g/dl	2.9	12.5	8.08
Platelet x 10(9)/L	7	405	83.8
Post remission induction chemotherapy (Day 28)			
WBC x 10(9)/L	1.0	16.1	6.2

WBC – White blood cells, Hb – haemoglobin,

Table 3: Minimal residual disease (MRD) status of precursor B-ALL at day-28 post-induction chemotherapy and during continuation of chemotherapy (week-12 and week-20).

MRD status	MRD at end of induction (Day-28)	MRD during continuation therapy	
		Week-12	Week-20
Negative	19	25	23
Positive	11	11	3
ND / NA	8	2	12

ND – not done; NA – not available

highest count being 322.0x10^9/L (Table 2) and the lowest WBC was 2.0x10^9/L. The mean WBC was 29.07x10^9/L. Most of the patients showed significant reduction in the WBC at day-28 post chemotherapy.

Residual leukaemic cells by flow cytometry (Table 3)

Residual leukaemic cells (MRD) had been analyzed by flow cytometry at the specified points of time as mentioned earlier. However, only 30 cases at day-28 of chemotherapy were available for MRD analysis, where 11 cases were found to be positive for MRD and 19 cases had no residual disease. At week-12 of therapy, two cases had to be excluded due to inadequate marrow samples for MRD analysis but the number of cases that became negative for MRD had increased to 25 patients (69.0 %).

However, at week-20, fewer samples were available for the analysis. There were inadequate specimens in ten patients, one patient had defaulted the follow-up treatment and sought traditional medicine while another had succumbed to sepsis.

Correlation between demographic, clinical and laboratory data of ALL cases at diagnosis and the residual disease (MRD) at day-28 of therapy

There were only 30 cases for MRD assessment at day-28 of chemotherapy (Table 4). There was no correlation between sex, race or age of the patients at diagnosis with MRD findings at day-28 ($p>0.05$). There was no correlation between lymphadenopathy and hepatosplenomegaly at diagnosis with MRD at day-28 ($p>0.05$). Correlation between WBC, haemoglobin (Hb) and platelet counts at diagnosis with MRD at day-28 were also not significant ($p>0.05$). Cases with a haemoglobin count of less than 8g/dl and WBC of less than 50.0 x 109/L were negative for MRD. There was also no correlation between the immunophenotype of the blast cells and presence of myeloid aberrant markers at diagnosis with MRD at day-28 ($p>0.05$).

Correlation between morphological and flowcytometric assessments of residual disease (MRD) of ALL cases

In this study, 35 cases achieved complete marrow remission at day-28 of induction chemotherapy by morphological examination. One case (Table 6; case 6) had a borderline blast cells percentage of 5%. Analyses for MRD by flow cytometry at day-28 could be performed in only 30 cases and 11 cases were found to be positive for MRD (Table 5) but the morphological examination of their marrow specimens showed blast counts of less than 5%.

For the week-12 analysis, bone marrow aspirations could be performed only on 36 of 38 cases and the correlation between marrow findings and MRD at

Table 4: Correlation between the MRD status (at day-28 post-induction chemotherapy) and features of childhood ALL cases at diagnosis

	Number of cases	%	MRD at day-28		P
			positive	negative	
Age (year)					
< 2 years	5	16.7	4	1	0.074
2 - 10 years	24	80.0	7	17	
> 10 years	1	3.3		1	
Sex					
Male	23	76.7	9	14	0.612
Female	7	23.3	2	5	
Race					
Malay	24	80.0	11	13	0.114
Chinese	4	13.3	0	4	
Indian	2	6.7	0	2	
Immunophenotyping					
Common ALL	26	86.7	10	16	0.603
Null	4	13.3	1	3	
Aberrant myeloid antigen					
present	17	56.7	5	12	0.346
absent	13	43.3	6	7	
Lymph nodes					
present	23	76.7	10	13	0.161
absent	7	23.3	1	6	
Liver					
≥ 5 cm	8	26.7	2	6	0.424
< 5 cm	22	73.3	9	13	
Spleen					
≥ 5 cm	6	20.0	3	3	0.449
< 5 cm	24	80.0	8	16	
Haemoglobin (Hb) at diagnosis					
< 8 g/dl	18	60.0	6	12	0.750
8 - 10 g/dl	6	20.0	2	4	
> 10 g/dl	6	20.0	3	3	
White cell count (WBC) at diagnosis (x 10^9/L)					
< 50,000	28	93.3	11	17	0.265
≥ 50,000	2	6.7	0	2	
Platelet count at diagnosis (x 10^9/L)					
< 50,000	15	50.0	6	9	0.720
50,000 - 100,000	8	26.7	2	6	
> 100,000	7	23.3	3	4	

Table 5: Correlation between bone marrow (BM) morphology findings and status of minimal residual disease (MRD) at day-28 and week-12 post-induction chemotherapy.

MRD	BM morphology at day 28		BM morphology at week 12	
	Blast < 5%	Blast > 5%	Blast < 5%	Blast > 5%
Negative	19	0	25	0
Positive	11	0	9	2
Total cases	30	0	34	2
P			0.028	

week-12 was found to be statistically significant ($p<0.05$). Flow cytometric analysis at week-12 showed that there were nine cases with positive MRD although their morphological marrow assessments showed that the percentages of the blast cells were less than 5% (Table 5). One of the cases with positive MRD (case 6) had also not achieved complete marrow remission at day-28 (Table 6). Two cases were found to have blast cells of more than 5% of total nucleated cells by morphological examination and they were also positive for MRD by flow cytometric analysis.

Sequential determination of residual disease and survival

There were only 3 cases (Case 1, 3 and 6) with persistent MRD at week-20 of chemotherapy. These cases however, had no MRD assessment at day-28 (Table 6). Case 1 and 3 had marrow remission morphologically at day-28 but case 6 had borderline blast cell percentage (5%). Case 1 relapsed after one year while case 3 after six months of treatment. Both succumbed to treatment complications. To date, case 6 is still alive, which is more than 5 years after diagnosis.

There were four cases with persistent MRD from day-28 until week-12 of therapy but at week-20, three cases achieved a negative MRD status. However, MRD analysis could not be done on one case (case 38) due to insufficient sample. To date, only one patient (case 5) had passed away after 3 years of diagnosis.

There were 18 cases that were always negative for MRD. However, eight of them died within 12 weeks to 4 years after diagnosis. Case 8 had relapsed after 3 years of treatment; case 39 had defaulted and relapsed while others remained in remission but unfortunately had succumbed to sepsis. Twenty four patients were still alive after 5 years of follow up.

DISCUSSION

Acute lymphoblastic leukaemia (ALL) is the most common malignant disease-affecting children and in Malaysia approximately 75% of ALL cases are younger than 20 years of age (Lim et al. 2002). Most of the cases in this study were in the good prognostic age group i.e. two to ten years of age (73.7%) but majority of the cases were males (81%). A longer survival of cases in age group between two and ten years have been reported, which was more than one-and-a-half times as compared to patients with age of less than two years or more than 10 years, when treated in an identical manner (Pui et al. 2008).

Gender has little or no impact on the success of remission induction and the relapse frequency during the first month of treatment. However, after discontinua-

Table 6: Bone Marrow morphology and MRD status at post-induction chemotherapy, during continuation of therapy and survival status after 5 years.

Patient no.	Marrow morphology at day-28	Marrow morphology at week-12	MRD Status			MRD markers	Patient status after 5 years of diagnosis
			Day-28	Week-12	Week-20		
1	blast < 5%	blast > 5%	-	Positive	Positive	TdT	relapse /died
2	blast < 5%	blast < 5%	-	Negative	Negative	CD33/10	alive
3	blast < 5%	blast > 5%	-	Positive	Positive	TdT	died
5	blast < 5%	blast < 5%	Positive	Positive	Negative	TdT	died
6	blast = 5%	blast < 5%	-	Positive	Positive	TdT	alive
7	blast < 5%	blast < 5%	-	Positive	Negative	CD19/34	alive
8	blast < 5%	blast < 5%	Negative	Negative	Negative	TdT	relapse/died
9	blast < 5%	blast < 5%	Positive	Negative	Negative	TdT	alive
10	blast < 5%	blast < 5%	Negative	Negative	Negative	TdT	alive
11	blast < 5%	blast < 5%	Negative	Negative	Negative	CD19/34	died
12	blast < 5%	blast < 5%	Positive	Positive	Negative	CD19/34	alive
13	blast < 5%	blast < 5%	Positive	Negative	Negative	CD19/34	alive
14	blast < 5%	blast < 5%	Positive	Positive	Negative	TdT	alive
15	blast < 5%	blast < 5%	Positive	Negative	Negative	CD33/10	alive
16	blast < 5%	blast < 5%	Negative	Negative	Negative	CD19/34	alive
17	blast < 5%	blast < 5%	Negative	Negative	Negative	CD33/10	alive
18	blast < 5%	blast < 5%	Negative	Negative	Negative	CD33/10	died
19	blast < 5%	blast < 5%	Negative	Negative	Negative	CD19/34	alive
20	blast < 5%	blast < 5%	Positive	Negative	Negative	CD33/10	alive
21	blast < 5%	blast < 5%	Positive	Negative	Negative	CD19/34	alive
22	blast < 5%	blast < 5%	Negative	Negative	Negative	CD33/10	alive
26	blast < 5%	blast < 5%	Positive	Negative	Negative	CD19/34	alive
28	blast < 5%	blast < 5%	Negative	Negative	Negative	CD19/34	alive
29	blast < 5%	blast < 5%	Negative	Negative	Negative	CD19/34	alive
31	blast < 5%	blast < 5%	Positive	Negative	-	CD19/34	unknown
33	blast < 5%	blast < 5%	Negative	Negative	-	TdT	alive
34	blast < 5%	Inadequate	Negative	-	-	CD19/34	died
35	inadequate.	blast < 5%	-	Positive	Negative	CD19/34	CNS relapsed/died
37	.inadequate	blast < 5%	-	Positive	-	CD19/34	alive
38	blast < 5%	blast < 5%	Positive	Positive	-	CD19/34	alive
39	blast < 5%	blast < 5%	Negative	Negative	-	CD19/34	Defaulted/died
40	blast < 5%	blast < 5%	Negative	Negative	-	TdT	alive
42	blast < 5%	blast < 5%	Negative	Negative	-	CD33/19	Gullain Barre/Chronic paralysis/died
43	blast < 5%	blast < 5%	Negative	Negative	-	TdT	died
44	Blast < 5%	blast < 5%	-	Positive	Negative	TdT	Relapsed and refused treatment/died
46	blast < 5%	blast < 5%	Negative	Negative	-	CD19/13	alive
47	blast < 5%	blast < 5%	Negative	Negative	-	CD33/10	died
49	blast < 5%	inadequate	Negative	-	-	TdT	alive

* (-) indicate no sample available.

tion of therapy, boys continue to experience a higher incidence of relapse due to relapse in the testes as well as in the bone marrow (Chessells et al. 1995). In this study, 12 of 31 (38.7%) male patients had relapsed and died within five

years. The number of female patients involved in this study was small for a comparative study.

Ethnically, the majority (78.9%) of the cases were Malays, followed by Chinese (13.2%) and Indians (7.9%). This observation is consistent with the data of the National Cancer Registry published in 2002, where Malays have the highest incidence of leukaemias (Lim et al. 2002).

The sizes of peripheral lymph nodes, liver and spleen provide an indirect measurement of leukaemic cell burden. Several studies have demonstrated that massive lymphadenopathy; hepatomegaly and splenomegaly adversely affect remission duration and survival (Ojala et al. 1995). However, mild to moderate increases in the size of lymph nodes and abdominal organs do not appear to influence prognosis of ALL cases. In this study, most of the cases presented with lymphadenopathy (76.3%), hepatomegaly (89.5%) and splenomegaly (76.3%), and enlarged liver and spleen of 5.0 cm or more were seen in 11 cases (28%) and 8 cases (21%) respectively.

The total white blood cell count (WBC) at the time of diagnosis is the single most powerful determinant of remission induction, remission duration, and long-term survival for all age groups (Pui et al. 2008). Patients with higher WBC at diagnosis have a higher risk for treatment failure than patients with lower WBC, and often have bulky extramedullary disease. WBC of more than 50.0 x 10^9/L is an indication of poor prognosis (Pui et al. 1998; Shu & Chen 2005).

In this study, the clinical and haematological characteristics of children with ALL at diagnosis were compared with the residual disease status detected by flow cytometry, to assess for any correlation. MRD detection on completion of induction therapy was not significantly related to age, gender, race, lymphadenopathy and hepatosplenomegaly, leukocyte count, haemoglobin level, platelet count, immunophenotype of lymphoblast, and presence or absence of myeloid aberrant markers. It appears that the above factors could not be used to predict MRD status at day-28.

Early clearance of leukaemic cells is a favourable prognostic indicator in childhood ALL. However, identification of residual leukaemic cells by light microscopy is subjective and lacks sensitivity. Common therapeutic protocols have considered 5% of blast cells in the bone marrow as the morphologic limit for considering complete remission of ALL patients. Even though intensification of chemotherapy, risk stratification and supportive care have improved the outcome of childhood ALL, 20% of children still succumb to relapse. Thus, a patient declared to be in complete clinical remission might, harbour as many as 10^{10} leukaemic cells. The study of MRD has improved the estimation of the number of blast cells present in the bone marrow during complete remission and during or after therapy. Thus it may be helpful to design a risk-adapted therapy in patients with acute leukaemia. The ability to detect residual leukaemic cells while they are still sensitive to treatment would allow for potentially curative intervention. The persistence of small numbers of leukaemic cells post induction chemotherapy in childhood ALL, indicates the requirement of prolonged therapy (2.5 to 3 years) to reduce the relapse hazard (Pui & Crist 1994; Hoelzer 1994).

The sensitivity of MRD detection using flow cytometry depends on the numbers of cells analyzed and also on the MRD markers used. For optimal flow cytometry results it is imperative that the bone marrow specimen contain a large number of viable with a good separation of mononuclear cells. The sample preparation and live gate acquisition should be performed within 24 hours. If the samples have remained unprocessed for a longer

duration, there will be changes in the FSC/SSC pattern and the increasing population of dying or apoptotic cells will interfere with the analysis and interpretation of the results. Post chemotherapy bone marrow samples also have a large number of new regenerating normal lymphoid cells (haematogones) which may express CD34, CD19, CD10 and TdT and may affect the analysis of results (Coustan-Smith et al. 2006).

We had used a two-color flow-cytometry for measurement of MRD. Patients with a high tumour burden at diagnosis and have MRD more than 1% post chemotherapy are more likely to relapse early (Coustan-Smith et al. 2000). In this study, there were three cases with persistent MRD positivity and the correlation study between marrow findings and MRD status at week-12 was found to be statistically significant ($p<0.05$). Two of these patients had an early relapse and succumbed to the disease. Marrow remission status post chemotherapy of patients with MRD less than 1% were questionable (Coustan-Smith et al. 2000). There were 18 cases in this study that had not shown MRD positivity at any point of time but eight of them died within a period of 12 weeks to 4 years after diagnosis.

Coustan-Smith et al. (2006) showed that by using multi-coloured flow-cytometry, MRD can be detected up to 0.01% and the cases that showed less than 0.01% MRD positivity at the end of remission induction are likely to have an excellent treatment outcome, compared to those with more than 0.01% (Coustan-Smith et al. 2006).

In the Paediatric Haemato-Oncology Unit UKMMC, stratification of intensive treatment usually depends on the presenting WBC and age of the patient. Following induction chemotherapy, patients who had more than 5% of blast cells in the bone marrow morphologically and patients with nervous system (CNS) or testicular relapse will be given a more intensive chemotherapy regimen. Thus MRD analysis by flow cytometry can also be used to design a risk-adapted therapy in childhood ALL. The ability to detect residual leukaemic cells while they are still sensitive to treatment would allow more time, and potentially curative intervention. The persistence of small numbers of leukaemic cells post induction chemotherapy in childhood ALL indicate the requirement of prolonged therapy (2.5 to 3 years) to reduce the relapse hazard (Campana 2003; Hoelzer 1994).

This study was unable to prove that the risk of relapse was high in patients with persistent MRD since a few cases which were MRD negative also relapsed within 4 years after diagnosis.

CONCLUSION

This study showed that there was no significant correlation between MRD positivity by flow cytometry with the demographic, clinical or haematological data at diagnosis. However, there was a significant correlation between MRD positivity by flow cytometry with morphological analysis of blast cells by light microscopy. For better evaluation of MRD, a three or four colour flow cytometry analysis should be used and a larger cohort of ALL cases are required, and would then aid in providing a basis for future clinical decision making in the management of ALL cases.

ACKNOWLEDGEMENT

The authors gratefully acknowledge and thank Mrs Sivagengei for her technical assistance and advice regarding flow cytometry.

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