

Biphenotypic hybrid acute leukaemia detected by two colour flow cytometry

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Abstract

Immunophenotypic studies using immunofluorescent flow cytometry were performed on the blast cells of 36 patients with acute leukaemia using a panel of eight monoclonal antibodies. Six patients had blasts which co-expressed markers for lymphoid and myeloid differentiation, and which were therefore defined as biphenotypic hybrid acute leukaemia. Of the six, three patients were in the paediatric age group (below 12 years old) while the other three were more than 12 years old. Peripheral blood counts were variable; however, bone marrow infiltration was extensive (blasts 275% in all). At the time of study, remission was achieved in only two patients. The authors' data show that biphenotypic hybrid acute leukaemia is not rare in Malaysia. This represents a subgroup of acute leukaemia identifiable by immunophenotyping but not by the French-American-British classification based on morphological and basic cytochemical studies alone. The recognition of this subgroup is important for both practical and theoretical reasons. There are implications for treatment of the individual patient because treatment directed at a single lineage may not be effective. The two colour flow cytometry proved to be a useful tool for diagnosis and classification of acute leukaemia.

Key words: Leukaemia, flow cytometry.

INTRODUCTION

Acute lymphoblastic leukaemia (ALL) and acute myeloid leukaemia (AML) are haematologic malignancies characterised by proliferation and accumulation of immature haemopoietic cells and their progenitors. The distinction between ALL and AML has been based mainly on morphological and cytochemical features. Recent advances in immunophenotyping have demonstrated that a significant sizeable minority of acute leukaemias have both lymphoid and myeloid features. Numerous terms are used to refer to these leukaemias including hybrid, biphenotypic, biclonal, simultaneous, bilineal, synchronous, metachronous, chimaeric, mixed, lineage switch and others. Gale and Ben-Bassat² designated these cases as hybrid acute leukaemia and proposed stringent diagnostic criteria. According to those criteria, hybrid acute leukaemia can be classified into two subgroups. One is 'biphenotypic' leukaemia in which both lymphoid and myeloid markers are co-expressed in >10% of blasts; the other is 'bilineal' leukaemia in which the blasts are heterogenous with single cells displaying either lymphoid or myeloid features but not both. We describe here features of

biphenotypic hybrid acute leukaemia detected by two colour immunofluorescent flow cytometry.

MATERIALS AND METHODS

Between 1st April, 1991 and 31st October, 1991, fresh leukaemic bone marrow or peripheral blood (>50% blasts) from thirty-six patients with acute leukaemia from the University Hospital, Kuala Lumpur and General Hospital, Kuala Lumpur were immunophenotyped. Six patients had biphenotypic hybrid acute leukaemia.

Morphological and cytochemical analysis

Bone marrow smears were stained by standard techniques including May-Grunwald-Geimsa (MGG), myeloperoxidase (MPO), periodic acid-Schiff (PAS) and, in some cases, alpha-naphthol acetate and chloroacetate esterases and acid phosphatase (AP). By standard convention, when >3% blasts reacted the cytochemical test was considered positive. The diagnosis of acute leukaemia based on the French-American-British (FAB) classification³ was made without knowledge of the immunophenotyping results.

Immunophenotyping

Bone marrow or peripheral blood samples were collected in EDTA tubes. Monoclonal antibodies conjugated with fluorescein were added to the samples which were then incubated at room temperature for 15 minutes. The red blood cells were lysed by adding Faclyse lysing solution (Becton Dickinson). The samples were then spun, washed in phosphate buffered saline and respun before fixing in 1% formaldehyde. The samples were analysed using Facscan flow cytometer (Becton Dickinson). All cases were analysed using monoclonal antibodies directed against B-cell-associated antigens (CD10, CD19 and CD22), T-cell-associated antigens (CD2, CD3 and CD7) and myeloid-associated antigens (CD13 and CD33). The details on the antibodies used are given in Table 1. When single colour immunofluorescence analysis revealed expression of both myeloid- and lymphoid-associated antigens, dual fluorescence (fluorescein isothiocyanate - FITC and phycoerythrin - PE) analysis was performed. Mouse anti-human IgG₁FITC with IgG_{2a}PE (Simultest Control, Becton Dickinson) was used as negative control. For this study, leukaemia in which more than 30% of cells expressed both lymphoid and myeloid antigens were classified as biphenotypic hybrid acute leukaemia.

TABLE 1: Monoclonal antibodies used

Antibody	Cluster designation	Predominant reactivity	Source
Leu 5b	CD 2	E rosette receptor	BD
Leu 4	CD 3	T cell receptor complex	BD
Leu 9	CD 7	T cells, NK cells	BD
Calla	CD 10	Common ALL Pre B cells	BD
Leu M7	CD 13	Monocytes, granulocytes, AML	BD
Leu 12	CD 19	B cells	BD
Leu 14	CD 22	B cells	BD
Leu M9	CD 33	Monocytes, granulocytes, AML	BD

BD= Becton Dickinson

Treatment

Patients 1,3,4 and 5 received vincristine, daunorubicin and prednisolone for induction of remission. In addition, patients 1 and 4 also received intrathecal methotrexate. Patient 2 received prednisolone, ara-C and VP-16 while patient 6 received vincristine, daunorubicin, ara-C and prednisolone.

RESULTS

Six out of 36 patients immunophenotyped had blast cells expressing both lymphoid- and myeloid-associated antigens in >30% of cells. Three patients were in the paediatric age group (below 12 years old) while the other three were older. There were 4 males and 2 females. The presenting features and haematological data are summarised in Table 2. Peripheral blood counts were variable, however, and bone marrow infiltration heavy (275% blasts in all cases). The reactivity pattern of the blast cells is shown in Table 3. On the basis of the FAB classification, 3 patients had L2; 1 L1; 1 M2 and 1 M4 morphology. Four patients had blasts positive for B-lymphoid- (CD10, CD19 and/or CD22) and myeloid-associated antigens (CD13 or CD33) while two patients had blasts positive for T-lymphoid- (CD2, CD3 or CD7) and myeloid-associated antigens. Table 4 shows the results of two colour immunofluorescence analysis for confirming the co-expression of myeloid and lymphoid antigens.

Following induction therapy (Table 5), two out of the three adult patients went into remission. Three patients (1 adult and 2 children) were not in remission despite being given chemotherapy for a duration of between 5 to 15 weeks. There was no follow-up data available for patient 2 as he discharged himself at his own risk.

DISCUSSION

There is a certain degree of disagreement about the frequency of hybrid acute leukaemia. The incidences reported vary (in different series)^{4,5} from 4% to 33% of all cases of acute leukaemia. This wide variation is due to the use of different panels of cell markers, immunophenotyping techniques, and diagnostic criteria.⁶ The true incidence of biphenotypic hybrid acute leukaemia in Malaysia is hard to ascertain as a large number of acute leukaemia are not phenotyped routinely once the diagnosis of ALL or AML is established using the FAB classification. Our limited data show that biphenotypic hybrid acute leukaemia is not rare in Malaysia. This represents a subgroup

TABLE 2: Clinical and haematological data of patients

Case	Age (yrs)/ Sex	Symptoms	Signs					Full blood count		
			Spleen (cm)	Liver (cm)	Lymphadenopathy	Bruises	Others	Hb (g/dl)	TWBC (x10 ⁹ /L)	Platelet (x10 ⁹ /L)
1	4/M	Anaemia	9	7	t	t	0	3.1	8.2 (Blast 80%)	15
2	7/M	Anaemia Fever	2	5	+	t	0	4.8	218.5 (Blast 70%)	212
3	25/M	Anaemia Neck swelling	Tipped	0	t	0	0	5.7	278.4 (Blast 95%)	49
4	2/F	Anaemia Fever	3	7	t	t	0	8.9	10.4 (Blast 25%)	60
5	22/M	Anaemia Fever	0	3	0	0	0	7.2	889.2 (Blast 60%)	176
6	16/F	Mouth ulcers Calf pain	Tipped	3	0	+	Gum hypertrophy	14.4	62 (Blast 83%)	280

TABLE 3: Reactivity pattern of blast cells

Case	Marrow blast (%)	FAB type	Cytochemistry					Surface markers (% positive)							
			MPO	PAS	NSE	CAE	AP	CD10	CD19	CD22	CD2	CD3	CD7	CD13	CD33
1	80	L2	-	t (fine granular)	ND	ND	ND	76	85	74	12	11	12	78	46
2	75	M2	+	-	-	+	ND	58	87	25	6	5	9	87	33
3	90	L2	-	-	ND	ND	+	0	0	1	93	56	99	39	42
4	90	L1	-	t (coarse granular)	ND	ND	ND	93	96	97	2	1	1	64	47
5	90	L2	-	-	ND	ND	+	0	1	0	16	97	99	93	13
6	85	M4	+	-	+	+	ND	80	83	81	10	10	9	80	80

MPO : Myeloperoxidase
 PAS : Periodic acid-Schiff
 CAE : Chlorocetate esterase

NAE : Non specific esterase
 AP : Acid phosphatase
 ND : Not done

TABLE 4: Two colour immunofluorescence analysis

Dual positive surface markers according to patient No. (% of dual-labelled cells)					
1	2	3	4	5	6
CD10/CD13 (74)	CD10/CD13 (50)	CD7/CD13 (36)	CD10/CD13 (60)	CD7/CD13 (90)	CD10/CD13 (74)

TABLE 5: Response to induction therapy

Case	Clinical status at 31/10/91	Time to remission (weeks)	Duration of induction therapy at 31/10/1991 (weeks)
1	Not in remission yet	-	15
2	A.O.R.	?	?
3	Remission	6.5	6.5
4	Not in remission yet	-	6.5
5	Not in remission yet	-	5
6	Remission	4	4

A.O.R. = Discharged at own risk

of leukaemia identifiable by immunophenotyping but not by conventional morphological and basic cytochemical studies alone. The recognition of this subgroup of leukaemia is important for both practical and theoretical reasons. There are implications for treatment of the individual patient because treatment directed at a single lineage may be ineffective. We believe that many cases are not detected because phenotyping is not done routinely in Malaysia. The accurate diagnosis of hybrid acute leukaemia requires a broad and ever-expanding panel of markers, including analysis of gene rearrangements² and cytogenetic studies. We found the two colour flow cytometry to be very useful for directly demonstrating biphenotypic hybrid acute leukaemia especially when bone marrow samples contain residual normal marrow elements. Normal elements can complicate single-fluorescence analysis because staining of the leukaemic cells cannot always be distinguished from that of normal marrow cells expressing myeloid antigen.

Previous studies on response to chemotherapy in hybrid acute leukaemia have given conflicting results. While some series^{5,8,9,10} showed adverse prognostic significance, others^{6,7} showed no prognostic significance at all. The initial treatment outcome in our patients of the paediatric age group is poor. However, the follow-up period in our patients is too short to determine the long term outcome.

Further investigations in more cases should be undertaken to establish future classification of disease subgroups which can better indicate clinical and biologic importance.

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