

Philadelphia Positive Acute Lymphoblastic Leukaemia (ALL): Clinico-Haematologic Characteristics, Molecular Analyses and 3-year Follow Up—A Single Institution Study

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Abstract

Introduction: The Philadelphia chromosome is one of the commonest chromosomal aberration in adult acute lymphoblastic leukaemia (ALL) patients. We present the results of a 3-year prospective study to look at the clinico-haematologic, immunophenotypic, cytogenetic and molecular profile of 13 adult patients with Philadelphia (Ph) positive ALL out of 35 newly diagnosed ALL seen at our institution over the past 3 years. **Materials and Methods:** Thirty-five adult ALL patients seen between 1996 and 1998 comprised the study group. Marrow samples were obtained for immunophenotyping and karyotypic analysis at diagnosis. Samples were also obtained simultaneously for molecular testing for Ph chromosome. **Results:** Thirteen patients were found to be Ph positive by molecular analysis while cytogenetic studies identified the chromosomal abnormality in 9 of these patients. The median age of our Ph positive patients was similar to those without Ph chromosome. Pre-B phenotype appears to be common in this group of patients. In concordance with other studies, Ph positive ALL was associated with a poor prognosis in our patients. **Conclusion:** Identification of Ph chromosome is important in the management of patients with ALL as it is an important prognostic marker.

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Introduction

A number of specific chromosome abnormalities are associated with acute lymphoblastic leukaemia (ALL). The commonest in adults with B lineage ALL is the Philadelphia (Ph) chromosome t(9;22)(q34;q11).¹⁻³ This is found in 20% to 25% of adult ALL patients and has been found to predict for a poor prognosis.⁴ At the molecular level, the Ph chromosome corresponds to a fusion of the proto-oncogene c-Abelson (9q34) and the breakpoint cluster region (BCR) on band q11 of chromosome 22. Detection of the Ph chromosome can be carried out by two methods—karyotypic analysis or polymerase chain reaction (PCR) analysis of the breakpoint fusion region. Two types of BCR-ABL fusion genes are found in ALL patients by PCR analysis—the p210-type BCR-ABL gene that is commonly seen in chronic myeloid leukaemia patients and the p190-type BCR-ABL gene. The latter arises from the fusion of

BCR exon e1 to ABL exon a2 (e1a2 transcript) while the p210-type is a result of a fusion of either BCR exon b2 or b3 to ABL exon a2 (b2a2 or b3a2 transcripts).

Using the multiplex PCR to detect for the BCR-ABL transcripts,⁵ we looked at 35 consecutive cases of newly diagnosed ALL seen at our institution from January 1996 to December 1998. The clinico-haematologic, cytogenetic and molecular analysis results of 13 patients identified to be Ph positive are presented. In addition, we compared the use of conventional cytogenetics with PCR for detection of Philadelphia chromosome at diagnosis.

Patients and Methods

Between 1996 and 1998, 35 patients with ALL age more than 12 years old (Table I) were seen in our institution. Patients with preceding history of chronic myeloid leukaemia were excluded from the study.

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TABLE I: COMPARISON OF CLINICO-HAEMATOLOGIC FEATURES OF Ph+ WITH NON-Ph+ ALL

	Ph+ ALL	Non Ph+ ALL	P value
No. of patients	13 (37%)	22 (63%)	-
Median age (years)	36.5	32	$P = 0.727$ (Mann-Whitney U test)
Age >50 years	3	6	$P = 0.712$ (Fisher's Exact test)
Age range (years)	(16-74)	(13-81)	-
Sex (male:female)	7:6	12:10	-
Race	12 Chinese 1 Eurasian	17 Chinese 3 Indians 2 Malays	- - -
WBC count	>50,000/ μ L >100,000/ μ L	7 (33%) 5 (24%)	$P = 0.187$ (Fisher's exact test) $P = 0.283$ (Fisher's exact test)
FAB morphological and cytochemical criteria			$P = 0.475$ (Pearson chi-square test)
L1	7 (54%)	13 (59%)	
L2	6 (43%)	6 (29%)	
L3	0	2 (10%)	
Others	-	eosinophilic ALL	
Phenotype		- 1	$P = 0.071$ (Pearson chi-square test)
Not done	0	1	
Number evaluable	13/13	21/22	
Null	0	3 (15%)	
Pre-B	10 (77%)	8 (36%)	
Pre-B cALL	0	1 (5%)	
cALL	3 (21%)	3 (15%)	
Mature-B	0	1 (5%)	
T-ALL	0	5 (25%)	

ALL: acute lymphoblastic leukaemia; cALL: common acute lymphoblastic leukaemia; FAB: French-American-British; Ph: Philadelphia; T-ALL: T-acute lymphoblastic leukaemia; WBC: white blood count

The diagnosis of ALL was based on May-Grunwald-Giemsa smears of bone marrow aspirates and leukaemia cells were classified according to French-American-British (FAB)⁶ morphological and cytochemical criteria. Patients were defined as Ph positive if they had the t(9;22)(q34;q11) detected by cytogenetics and/or molecular studies. Immunophenotypic analysis was carried out in 34 of the 35 patients using the EPICS-PROFILE II flow cytometer (Coulter) to subtype the patients as null, pre-B, common (cALL), mature B- and T-cell (7). The initial screening was based on reactivity for HLA-DR, CD3, CD5, CD10, CD19, CD20, surface immunoglobulin, CD13, CD33 and CD 34. Subsequently, depending on the results obtained, positivity

for terminal deoxynucleotidase (Tdt), intra cytoplasmic immunoglobulin (cIg) and cytoplasmic CD3 were assessed. A cut-off point of 20% and above was used for positively reacting cells for CD10, CD13, CD33, HLA-DR and cIg, 10% of leukaemia clasts for Tdt and 30% for other markers. The classification of ALL subtype was based on guidelines established by the European Group for immunological characterisation of acute leukaemia.⁷

Cytogenetic examination was successfully performed on bone marrow samples of 33 patients using the G-banding technique.⁸ Where possible, 20 metaphase cells were analysed and chromosome abnormalities were classified according to the International System for Human Cytogenetic Nomenclature (ISCN) 1995.⁹

Multiplex Polymerase Chain Reaction

Reverse transcription polymerase chain reaction (RT-PCR) by multiplex PCR⁵ to detect the BCR-ABL transcripts was carried out simultaneously using peripheral blood and/or bone marrow samples. RNA was obtained from mononuclear cells, isolated by Ficoll Histopaque density gradient centrifugation, using the total RNA isolation kit (Promega, USA). Ten μ g of total RNA (in 10 μ L of H₂O) was reverse transcribed into cDNA by adding 11 μ L cDNA mix {95mM Tris pH 8.3, 142.5 mM KCL, 5.7 mM MgCl₂, 19 mM dTT, 1.9 mM each dNTP's, 200 μ g/mL random hexamers (Promega), 300U M-MLV reverse transcriptase (Promega), 32U Rnasin (Promega)} to samples and incubating at 37°C for 2 h. A reagent blank (10 μ L of H₂O) was included at this stage. The reaction was terminated by heating to 65°C for 10 min and the resultant cDNA stored at -20°C.⁵

A single multiplex PCR was carried out using 4 μ L of the above cDNA in a total volume of 21 μ L BCR-ABL mix (12 mM Tris pH 8.3, 1.8 mM MgCl₂, 60 mM KCl, 0.24 mM each dNTP's, 0.5U thermostable Taq DNA polymerase and 0.6 μ M primers: BCR-C (5'-accgcatgttccgggacaaaag-3'), B2B(5'-acagcattccgctgacatcaataag-3'), C5e-(5'-cagctctccttgcaaccgggtctgaa-3') and CA3-(5'-tggtgactggcgtgatgatgttagttgcttg-3').

PCR was performed on a 2400 Perkin Elmer programmable heating block by 35 cycles of 96°C 1 min, 64°C 50 s, 72°C 1 min followed by a 10-min extension at 72°C. To increase the specificity of the reaction, tubes were placed in the thermocycler only when it had reached the denaturing temperature. Reaction products were electrophoresed on a 2% ethidium bromide-stained agarose gel and then visualised directly under ultraviolet light and photographed. Bands of 481 bp were visualised for p190 e1a2 transcript, 385 bp for p210 b3a2 and 310 bp for p210 b2a2 transcripts, respectively. The RNA quality and cDNA preparation were evaluated by amplification of the normal

BCR mRNA in all specimens with primers B2B and C5e- (808 bp). The sensitivity of this technique was of the order of 10^{-3} .

Each PCR experiment included a blank (with all components of the PCR reaction mixture except RNA), negative control (with all components of the PCR reaction mixture and RNA from normal blood) and known positive controls. The analysis for all samples was repeated again by another technologist and also, where both blood and marrow samples were available from the patients, both were run on separate occasions.

Statistical Analysis

The clinico-haematologic features of Ph positive patients were compared with non Ph positive cases and *P* values were calculated with the SPSS statistics program.

Therapy

Of the 13 Ph positive ALL patients, 5 patients were treated with the L17 protocol¹⁰ while 5 were given the hyperCVAD protocol.¹¹ Marrow response status was assessed post induction chemotherapy by bone marrow aspirates on day 28. Complete remission was defined as less than 5% blasts in bone marrow aspirates with evidence of maturation of cell lines and restoration of peripheral blood counts. Three patients underwent bone marrow transplant—2 allogeneic transplants in first remission (patients 9 & 10) and 1 match unrelated transplant in refractory relapse (patient 8). Three patients opted for palliative chemotherapy.

Results

Study Population

There was no statistically significant difference in the median age (Table I) of the Ph positive patients compared with the Ph negative cohort. No difference was found in the incidence of patients aged 50 years or more between the two groups. Total white cell count of more than 50,000/ μ L was seen in 7 Ph positive patients at initial diagnosis and 7 patients in the Ph negative group. This was not found to be statistically significant. This was similarly so when we looked at the number of patients with presenting white cell count of more than 100,000/ μ L. Phenotypic analysis results suggest Ph positive cases are commonly of pre-B or common ALL phenotype.

Cytogenetics

Of the 35 patients, 13 were found to be Ph positive by PCR analysis (Table II). However, Ph chromosome was detected in only 9 patients by karyotyping. The main reason for non-detection of the Ph chromosome by cytogenetics is poor quality of metaphases. Variant Ph chromosome with 3-way translocation involving chromosomes 9, 12 and 22 (patient 5) and chromosomes 9, 15 and 22 (patient 6) were seen in 2 patients. Three patients (5, 9 and 10) had additional cytogenetic abnormalities to the Ph chromosome while 1 (patient 11) had only other abnormalities with no Ph chromosome.

Molecular Studies

Both blood and marrow samples were obtained from 28

TABLE II: PATIENT CHARACTERISTICS, RESPONSE TO THERAPY AND OUTCOME OF 13 Ph+ ALL PATIENTS

Patient no.	Age (y)/Sex	Phenotype	Molecular abnormality	Karyotype	Therapy	Response	CR duration (mo)	Survival (mo)
1	17/M	pre-B	p210 b2a2	Ph	L17	CR	5	9
2	34/F	pre-B	p210 b2a2	Ph	L17	CR	5	10
3	16/F	pre-B	p210 b3a2	Normal	L17	Refractory	NA	2
4	38/F	cALL	p210 b2a2	Ph	HyperCVAD	CR	16	17+*
5	41/F	cALL	p210 b2a2	Variant Ph and others t(3;5), -10, add 10p	HyperCVAD	CR	8	13
6	56/M	pre-B	p210 b3a2	Variant Ph	HyperCVAD	Refractory	NA	11
7	74/F	pre-B	p210 b2a2	No dividing cells	Palliation	NA	NA	3
8	16/M	pre-B	p190	Ph	L17(BMT in relapse)	CR	7	13
9	49/M	pre-B	p190	Ph and others +2, t(11;21)	L17(BMT)	CR	NA	5
10	21/M	pre-B	p190	Ph and others -3, -21, +7, (add 19q)	HyperCVAD (BMT)	CR	8	15
11	25/M	cALL	p190	No Ph & others (add 9q), (del 22q), (add 16q)	HyperCVAD	Refractory	NA	2
12	43/M	pre-B	p190	Ph	Palliation	NA	NA	2
13	71/M	pre-B	p190	Insufficient metaphases	Palliation	NA	NA	6

ALL: acute lymphoblastic leukaemia; BMT: bone marrow transplantation; cALL: common acute lymphoblastic leukaemia; CR: complete remission; NA: not applicable; Ph: philadelphia chromosome; +*: still alive; -: monosomy; +: trisomy

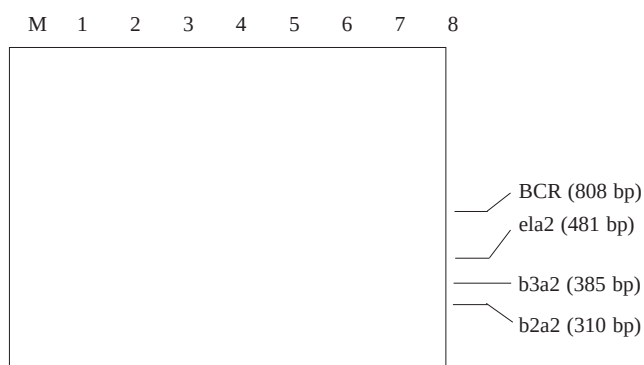


Fig. 1. Multiplex PCR results of 3 patients with Ph positive ALL. Lane M: pGEM DNA molecular weight marker; lanes 1-3: Ph positive ALL cases; lane 4: blank control; lane 5: normal patient; lanes 6-8: Ph positive controls

of the 35 patients simultaneously at diagnosis and results of the PCR analysis were completely concordant. Of the 13 Ph positive patients, only 6 (46%) possessed the p190 BCR-ABL fusion transcript (Table II) (Fig. 1). The majority (54%) had the p210 transcript with 5 patients having the p210 b2a2 type and 2 patients with p210 b3a2.

Response to Therapy

Ten of the Ph positive patients underwent induction chemotherapy with either L17 (5 patients) or hyperCVAD (5 patients). Four of the 5 patients receiving L17 went into complete remission while 3 patients from the hyperCVAD arm went into remission. However, 5 of the 7 patients who achieved remission subsequently relapsed. The duration of first remission was invariably short with relapses occurring within 1 year. To date, only 1 patient remains alive in complete haematological remission—patient 4 on hyperCVAD protocol who is still alive 17 months since initial diagnosis. Of the remaining patients who underwent bone marrow transplant, 1 (patient 10) subsequently relapsed post transplant and died of refractory disease while 2 (patients 8 and 9) died of transplant-related complications.

Discussion

The incidence of Ph positivity in adult ALL cases in our study (37%) is similar to that reported by other studies.^{2,12,13} Although some groups have found an increased prevalence of Ph with age,^{13,14} this was not confirmed in our study. There was no statistically significant difference in the median age of our Ph-positive patients when compared with the Ph-negative group (Table I).

Immunophenotypic analyses show that Ph positive ALL patients are mostly of B-cell phenotype as found in other series.^{11,12,15} More specifically, our findings suggest Ph-positive ALL are commonly of the pre-B or common phenotype. Similar to findings elsewhere, the p210 type of BCR-ABL protein predominated slightly in our group of

Ph-positive ALL patients.^{12,16-18} The dismal prognosis associated with Ph-positivity is reflected in the high number of relapses in our patients who achieved remission and the short duration of their remissions (range 5 to 16 months, median 7.5 months).

Thus, the Ph chromosome is commonly found in adult ALL and is associated with a poor prognosis. Early identification of this cohort of patients is important, especially for those in the younger age group who are eligible for allogeneic bone marrow transplant. To date, no chemotherapeutic protocol has been proven to be more effective than allogeneic transplant in effecting a cure for these patients. While PCR analysis is more sensitive than conventional cytogenetics in detection of the Ph chromosome,^{4,12} it may not be cost-effective to perform both at diagnosis. The main reason for the inability to detect Ph chromosome cytogenetically is poor quality of metaphases which is a common problem in karyotypic analysis of ALL cases. Recognition of the abnormal clones which may sometimes be multiple and complex requires skilled and trained cytogenetists, and even so, it may sometimes be difficult to characterise the clonal abnormality. In contrast, PCR is not dependent on dividing cells and is more specific as it is designed to detect the particular clonal abnormality. Thus in cases where cytogenetics fail or show normal karyotype and yet the patient has refractory disease, PCR analysis can be considered. For PCR analysis, either marrow or peripheral blood samples can be utilised. Samples can be obtained at diagnosis and subsequently processed should the cytogenetic analysis fail.

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