Prognostic significance of cell surface phenotype in acute lymphoblastic leukemia

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Abstract

Context: To find out the phenotypic character of lymphoblasts of acute lymphoblastic leukemia (ALL) patients in our study cohort and their possible effect on the prognosis. **Aims:** To investigate the phenotype in ALL in our demographic population and to prognosticate various upfront current protocols employed in our hospital. **Settings and Design:** The study spanned over a period of 4 years with retrospective and prospective data of January 2008 through December 2011. **Materials and Methods:** 159 patients of all age groups were enrolled for the study, of which flow cytometry was done in 144 patients. **Statistical Analysis Used:** Analysis was done using the variables on SPSS (statistical package for social sciences) software on computer. Survival curves were estimated by method of Kaplan-Meir. **Results:** Majority of the patients were of B-cell (68.1%) and 30.6% patients were of T-cell lineage. Of these, 80.6% patients were having cALLa positivity. Complete remission (CR) was achieved in 59.1%, 16.4% relapsed, and 20.1% patients died. **Conclusions:** Phenotyping has become an important and integral part of diagnosis, classification, management and prognosticating in ALL. B-cell has been found to have a better survival over T-cell lymphoblastic leukemia. cALLa antigen positivity has good impact in achieving CR in only B-cell lineage, myeloid coexpression has no significant effect on the outcome. BFM (Berlin-Frankfurt-Münster) based protocols though showed a higher CR and survival *vis-a-vis* UKALL-XII. However, patients enrolled in former group being of low risk category and lesser in numbers cannot be compared statistically with a fair degree of confidence.

Key words: Acute lymphoblastic leukemia, flow cytometry, immunophenotype, India, Kashmir, leukemia, phenotype, prognosis

Introduction

Acute lymphoblastic leukemia (ALL) is characterized by clonal proliferation, accumulation, and tissue infestation of neoplastic cells. It has bimodal distribution with initial peak at 1-4 years of age and second peak ≥60 years of age, a low but steady rise in incidence with increasing age.[1] Leukemic cells from patients of ALL express a variety of surface differentiation antigens which are also found on normal lymphocyte precursors at discrete stages of maturation. Therefore, ALL cells are thought to originate from normal lymphocyte precursors arrested at early stages of either B-cell or T-cell ontogeny. Majority of the leukemic cells express either B or T lineage differentiation antigen. However, a small fraction of the patients do express both T and B lineage antigens.

Previously, leukemias used to be diagnosed on the basis of morphology of the blasts. Though helpful, its accuracy in diagnosing acute leukemias was only around 80% and a significant number of patients were misdiagnosed and subjected to inadequate treatment, due to considerable overlap of morphological characters in myeloid and lymphoid cells.[2]

The sequence of events leading to the development of mature lymphoid cells is associated with cell surface modifications that can be easily analyzed by the use of monoclonal antibodies. Flow cytometry has increased the diagnostic accuracy to around 98%.[3] On this basis; patients can be divided into groups, depending upon the cell of origin like B-cell leukemia, T-cell leukemia. Further, B-cell leukemia can be subdivided into three categories early pre-B, pre-B, and mature B-cell leukemia.[4] Similarly, T-cell can be subdivided into pre-T cell and T-cell leukemia, according to stage of thymocyte differentiation receptor protein expression.[4] In addition, there are cells which express myeloid markers like CD-13 and CD-33 and is referred to as myeloid coexpression. The clinical significance of myeloid co expression has been widely debated.[5-13] The importance of the immunophenotypic analysis of ALL has been documented by numerous studies in adults and children.[14]

Despite recent progress in the management of ALL, prognosis is still guarded. If complete remission (CR) is achieved in 70%-80% of the patients, relapses occur frequently and long term disease survival does not exceed 25%-30% in most of the studies.[15-20] It has been seen that patients with lymphoblast's of T-cell lineage have a much poorer prognosis than those with a reciprocal subset of non-T-cell lymphoblasts.[21-23] Cytogenetics t(9:22), t(4:11), t(1:9), and MLL (Mixed Lineage Leukemia) gene rearrangements have their own impact on outcome.

The present study aimed to investigate the phenotype in ALL in our demographic population and to prognosticate various upfront current protocols employed in our hospital. Also, comparative analysis of relevant available data was done. Impact of markers to subtype leukemias and treat with fair degree of confidence is an established norm. This being first from our state and the institution encouraged us to share our phenotype scenario of leukemias. In fact, this is the first and only study available in North India, depicting significant heterogeneity in phenotypic expression of ALL in India. We believe this could reflect different genotype and biology of our population, opening a window of opportunity for studying therapeutic variability.

Materials and Methods

Patients

Newly diagnosed cases of all age groups were enrolled and followed. Further, patients who were already enrolled since January 2008 were recruited retrospectively. Their data were retrieved from hospital database and those with incomplete recorded data were kept out of the study. Therefore, a 4-year study spanning from January 2008 to December 2011. Informed consent was taken from the patients or their guardians as eligible. Patients with incomplete data or improper follow-up were kept out of the study.

Flow cytometry

Heparinised bone marrow samples were used for flow cytometry using a panel of monoclonal antibodies to analyze blast cell population. Classification was based on expression of basic leukemia panel like CD3, CD5, CD7, CD8, CD10, CD19, CD20, CD21, CD22, CD13, CD33, CD117, CD79a, HLA-DR, cytoplasm, and surface immunoglobulins. For the markers to be positive,

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minimum of 20% of gated blast cells had to express the receptor. Patients were sub classified into early pre-B, pre-B, mature B-cell, pre-T, T-cell, mixed phenotype leukemia, or biphenotypic acute leukemia, according to the previously set World Health Organization criteria and European Group for the Immunological Characterization of Leukemias (EGIL) scoring system.

Protocols

Patients were treated with different protocols like UKALL-XII, Modified BFM-90, pediatric BFM (intermediate and standard risk), and prophylactic cranial irradiation depending on defined risk category. High-risk patients and T-cell leukemias were treated usually with UKALL XII protocol. Also, prophylactic cranial irradiation was given in 65% cases.

Statistics

Analysis was done using the variables on SPSS (statistical package for social sciences) software on computer. Survival curves were estimated by method of Kaplan-Meir.

Results

General Immunophenotypic characteristics of the study population [Table 1]

As shown, immunophenotyping was done in 144 patients out of 159. According to the criteria set, 98/144 (68.1%) were of B-cell lineage, which was further sub classified into early Pre-B cell 53/98 (36.8%), pre-B cell 42/98 (29.2%), and mature B-cell 3/98 (2%).

Out of 144 patients, 44 belonged to the T-cell lineage (30.6%). Of these, pre-T cell was seen in 6/44 (4.2%) and T-cell was seen in 38/44 (26.4%).

Also, 94/98 patients (95.6%) in B-cell lineage expressed cALLa antigen while only 50% of the T-cell cases were cALLa positive.

Myeloid co expression (mixed phenotype acute leukemia) was seen in 14/144 cases (9.7%) and biphenotypic acute leukemia was found in 2/144 patients (1.4%).

Comparative analysis of major immunophenotypic groups [Table 2]

T-cell ALL had CR in only 17% and 83% of the cases in this group relapsed or died, which was statistically significant.

Table 1: Immunophenotype of the ALL cases in Kashmir

	N	%
T-ALL		
Pre T cell	6	4.2
T cell	38	26.4
Total	44	30.6
B-ALL		
Early pre-B cell	53	36.8
Pre-B cell	42	29.2
Mature B Cell ALL	3	2.1
Total	98	68.1
cALLa+ve	116	80.6
Mixed phenotype acute leukemia	14	9.7
Biphenotypic acute leukemia	2	1.4
cALLa+ve T cell	22	15.3
cALLa+ve B cell	94	65.3
IPT		
Done	144	90.6
Not done	15	9.4

IPT=Immunophenotyping, ALL=Acute lymphoblastic leukemia

cALLa antigen positivity had no significant impact on the outcome or prognosis of T-cell ALL cases.

Prognosis was significantly better in B-cell ALL group when compared with the T-cell group. Also, cALLa antigen positive B-cell cases had significantly more chances of achieving CR (76.6%) and lesser relapse with *P* value of 0.003.

Early pre-B cell leukemia was significantly associated with myeloid co expression ($P = 0.025$), whereas pre-B cell phenotype was negatively associated with myeloid co expression ($P = 0.012$). However, there was no significant effect on the prognosis due to myeloid co expression, neither was there any relation to cALLa antigen positivity.

Comparison of the protocol used to the prognosis [Table 3]

There was significant difference in slow early response (SER), CR, relapse, failure and death between different protocols used.

UKALL-XII

Around half of the patients were put on this protocol (49.9%). First marrow after induction in patients on this protocol showed that 41.4% had SER, whereas 58.6% had CR. Marrow examination after complete treatment revealed that CR was maintained in 41.4%, relapse in 22.9%, failure in 5.7%, and 28.6% patients died during the course of study. Overall survival rate was 71.4% at the end of the study.

Modified BFM 90 protocol

A total of 25.8% cases were put on this protocol. After induction, marrow showed CR in 84.2% and SER in 15.8% patients. At the end of study 71.1% maintained CR, 7.9% patients relapsed, and 21.1% cases died. Thus, overall survival at the end of study in this group was 78.9%.

Pediatric BFM (intermediate risk)

A total of 11.3% patients received this protocol. CR was achieved in 87.5% cases in first marrow after induction and same percentage was maintained till end of study. This rate was significantly higher than UKALL XII and Modified BFM 90 protocols. However, before making any generalizations, it should be noted that significantly less number of cases were on this protocol and that patients in other protocols were of high-risk category and were expected to have poor outcome.

Similarly, around 92.3% cases achieved and maintained CR on pediatric BFM (standard risk) but only a minority of patients were on this group and were of low risk category.

Mean follow up period of the cases was maximum of (23.0 ± 11.5) months in pediatric BFM (standard risk) and least in modified BFM-90 protocol (14.1 ± 9.5) months. There was no significant difference in the complications during treatment with different protocols.

Survival curves

The mean and median survival in age less than or equal to 18 years was 21 months each with standard error of 1, while that in the age group more than 18 years was 24 months respectively. There were more early deaths in the adult age group but was not statistically significant.

Male gender had slightly better survival than females but was not statistically significant.

Overall, UKALL XII protocol maintained high survival with a steady decline, whereas BFM-based protocols showed

Table 2: Effect of phenotype at presentation on outcome

	SER		CR		Relapse		Failure		Died		*P* value
	N	%	*N*	%	*N*	%	*N*	%	*N*	%	
Total T cell											
Yes	1	100.0	15	17.0	7	31.8	4	100.0	17	58.6	0.000 (Sig)
No	0	0	73	83.0	15	68.2	0	0	12	41.4	
Total B cell											
Yes	0	0	73	83.0	15	68.2	0	0	10	34.5	0.000 (Sig)
No	1	100.0	15	17.0	7	31.8	4	100.0	19	65.5	
Pre-T cell											
Yes	0	0	3	3.4	0	0	1	25.0	2	6.9	0.201 (NS)
No	1	100.0	85	96.6	22	100.0	3	75.0	27	93.1	
T cell											
Yes	1	100.0	12	13.6	7	31.8	3	75.0	15	51.7	0.000 (Sig)
No	0	0	76	86.4	15	68.2	1	25.0	14	48.3	
Early pre-B cell											
Yes	0	0	37	42.0	10	45.5	0	0	6	20.7	0.098 (NS)
No	1	100.0	51	58.0	12	54.5	4	100.0	23	79.3	
Pre-B cell											
Yes	0	0	33	37.5	5	22.7	0	0	4	13.8	0.069 (NS)
No	1	100.0	55	62.5	17	77.3	4	100.0	25	86.2	
Mature B cell ALL											
Yes	0	0	3	3.4	0	0	0	0	0	0	0.747 (NS)
No	1	100.0	85	96.6	22	100.0	4	100.0	29	100.0	

ALL=Acute lymphoblastic leukemia, CR=Complete remission, NS=Not significant, SER=Slow early response

Table 3: Protocols received and outcome in patients in whom immunophenotyping was done

	UKALL XII		Modified BFM-90		Paediatric BFM (intermediate risk)		Paediatric BFM (standard risk)		Others		*P* value
	N	%	*N*	%	*N*	%	*N*	%	*N*	%	
Ist marrow after induction											
Slow early response	29	41.4	6	15.8	2	12.5	1	7.7	1	16.7	0.003 (Sig)
Complete remission	41	58.6	32	84.2	14	87.5	12	92.3	5	83.3	
Final outcome at the end of study											
Slow early response	1	1.4	0	0	0	0	0	0	0	0	0.000 (Sig)
Complete remission	29	41.4	27	71.1	14	87.5	12	92.3	5	83.3	
Relapse	16	22.9	3	7.9	2	12.5	1	7.7			
Failure	4	5.7									
Died	20	28.6	8	21.1					1	16.7	
Overall survival											
Surviving	50	71.4	30	78.9	16	100.0	13	100.0	5	83.3	0.017 (Sig)
Died	20	28.6	8	21.1					1	16.7	

BFM=Berlin-Frankfurt-Münster

higher initial mortality but had subsequent better survival and tolerability.

Discussion

Impact of markers to subtype leukemias and treat with fair degree of confidence is an established norm. This being first from our state and the institution encouraged us to share our phenotype scenario of leukemias. Infact, this is the first and only study available in North India, depicting significant heterogeneity in phenotypic expression of ALL in India. We believe this could reflect different genotype and biology of our population, opening a window of opportunity for studying therapeutic variability.

A total of 69.8% of our study population was less than or equal to 18 years. F:M ratio in this group was 1:0.93, whereas it was 1:1.17 in those more than 18 years. Mean age of presentation was (15.9 ± 12.0) years. Majority of our cases were of B-cell lineage (68.1%), while T-cell leukemia was seen in 30.6% patients, which was higher than most Western data but comparable to South Indian studies. T-cell leukemias had poorer prognosis when compared with B-cell leukemia. cALLa positivity had significant bearing on CR in B-cell ALL but not in T-cell ALL. Myeloid co expression had no significant effect on prognosis. Biphenotypic acute leukemia was seen in 1.4% cases.

Majority of the patients were treated with UKALL XII protocol and the rest with BFM-based protocols. Patients on UKALL XII protocol maintained a high survival with a steady decline, whereas those on BFM-based protocols had higher initial deaths but better subsequent survival, exceeding significantly from that in UKALL XII protocol. There was no significant difference in the complications seen across different protocol groups. However, treatment interruption because of nutritional status and hepatitis did occur.

We conclude that phenotyping has become an important and integral part of diagnosis, classification, management, and prognosticating in ALL. B-cell lineage leukemia has been found

to have a better survival over T-cell lymphoblastic leukemia. While cALLa antigen positivity has good impact in achieving CR in only B-cell lineage, myeloid co expression has no significant effect on the outcome. BFM-based protocols though showed a higher CR and survival *vis-a-vis* UKALL-XII. However, patients enrolled in former group being of low-risk category and lesser in numbers cannot be compared statistically with a fair degree of confidence.

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