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BIOLOGY OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA IN INDIAN PATIENTS IS DIFFERENT FROM THE WEST

Biology of Childhood ALL

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Summary

The treatment outcome in childhood ALL in Indian patients has been reported to be poor as compared to 70 percent cure rate in the West. While this could be multifactorial in origin, one aspect could be a different biology of the disease affecting the treatment outcome. Collaborative studies between NCI, NIH, USA and three Indian centers, All India Institute of Medical Sciences, Tata Memorial Hospital and Cancer Hospital Chennai, revealed significant phenotypic and genotypic differences from the West. Thus, there was a high relative incidence of T Cell ALL, paucity of common ALL and absence of an early age peak. Molecularly, the frequency of chromosomal translocations studied by real time PCR revealed lower frequency of t (12; 21) which is associated with a good prognosis and more frequent translocations t (1;19) and t (9; 22) which are associated with a poor prognosis. It appears, therefore, that a different biology of childhood ALL in Indian patients contributes, at least in part, to the poor treatment outcome seen.

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Population based data from various Indian Cancer registries suggest that approximately 10,000 new cases of Acute Lymphoblastic Leukemia (ALL) occur each year. Most Indian publications have shown poor treatment outcome in these patients (1, 2, 3). This contrasts with present cure rates of over 70% in Western countries. This could be due to multiple factors in our region such as paucity of cancer treatment facilities, poor availability of drugs, poor compliance, lack of supportive therapy or poor hygienic conditions leading to infections and death. There is, however, reason to believe that the large difference in environments and in population genetics between developing countries and affluent nations would have corresponding differences in both the biology of ALL (phenotype and genotype) as well as in the patients themselves, e.g. with respect to drug metabolism, nutritional status and the presence of significant co-morbities such as hepatitis. These factors could potentially influence treatment efficiency and toxicity and, therefore, survival rates.

This article describes not only differences in patient characteristics from Western series but also the limited value of most widely used risk factors such as age and WBC count at presentation. It further describes the results of the immunophenotypic and molecular studies undertaken to characterize the biology of the disease in these patients.

The studies were initiated in an Indo-US collaborative project between the National Cancer Institute, NIH and three Indian institutions; All India Institute of Medical Sciences (AIIMS), Tata Memorial Hospital (TMH) and Cancer Institute, Chennai (CI) over several years.

Table-1 shows the outcome of treatment at the three Indian centers on a common therapy protocol (4). The protocol was based on standard treatment principles with a four drug induction (vincristine, prednisone, daunorubicin and asparaginase) followed by CNS prophylaxis with cranial radiation and intrathecal therapy. The study was open to patients aged 1 to 24 years old. Event Free Survival (EFS) was chosen as the

Table-1 : Outcome of Treatment at Three Indian Centers, 1990-1997

Centre	No. Patients	CR (%)	Toxic Deaths (%)	Relapses (%)	EFS at 4 yrs
TMH	652	94.8	10.6	28.8	60%
AIIMS	228	83.3	22.8	30.5	41%
CI	168	86.9	16.7	41.1	43%

most satisfactory primary endpoint to use as an overall indicator of efficacy and toxicity of treatment, since events included all deaths whether due to toxicity or disease progression.

Table-2 shows the patient characteristics at the three Indian centres between 1990 – 1997 and Table 3 the univariate and multivariate analysis of risk factors at AIIMS(4). Age could not be identified as a risk factor but WBC count was significatnt. Table 4 shows a comparison of WBCs in various series in India and the USA or Europe (4)

Immunophenotypic Studies

Immunophenotyping was done in all the three participating institutions. A much higher proportion of T cell cases was uniformly reported from all the three centers as compared to the Western figure of 15%. Cancer Institute, Chennai, reported 44% cases to express T cell markers (5). At AIIMS, it was 31.8% (6) and at TMH 21% (7). Interestingly, a similarly high percentage of T cell lineage ALLs (50%) in both children and adults, had also been reported from Egypt (8).

Molecular Studies

The four major chromosomal translocations observed in pre B-ALL in children include the t (12; 21), that results in the fusion of the TEL (ETV 6) and AML1 genes, t (1; 19), resulting in a chimeric protein of E2A and PB X 1, t (9;

Table-2 : Patient Characteristics at Three Indian Centers, 1990-1997

Characteristic	TMH	AIIMS	CI	P value
No Patients	652	228	168	-
Median Age	7.2	7.6	10	0.007
Pre B-ALL	75.2%	59.5%	45.4%	<0.0001
Pre T-ALL	20.7%	31.8%	43.1%	<0.0001
Other cell	4.1%	8.7%	11.5%	n/a
WBC[e] < 10,000/mm^3	44.0%	37.3%	39.9%	0.18
WBC 10 – 50,000[g]/mm^3	31.4%	31.6%	25.6%	
WBC 50 – 100,000/mm^3	10.0%	13.2%	11.3%	0.035
WBC > 100,000/mm^3	14.6%	18.0%	23.2%	0.023
Lymphadenopathy	74.2%	83.8%	72.0%	0.006
Hepatomegaly/splenomegaly	76.0%	89.5%	85.1%	<0.0001
Mediastinal mass, all patients	1.8%	7.8%	2.4%	0.0041
Mediastinal mass, pre-T cell patients	33.6%	46.8%	35.7%	0.21

Table-3 : Univariate and Multivariate Analysis of Risk Factors at AIIMS

	Univariate P-value			Multivariate P-values
Characteristic, Phenotype, or risk factor	**All Patients**	**Pre B-ALLb**	**Pre T-ALLc**	**All Patients**
Age	0.10	0.21	0.28	0.20
Sex	0.22	0.64	0.32	0.45
WBC count	0.0011	0.0025	0.95	0.0005
Blast count	0.073 n=218	0.052 n=113	0.45 n=59	0.39 n=208
Platelet count	0.041 n=218	0.0047 n=113	0.88 n=59	0.025
Phenotype	0.83 n=195	n/a	n/a	0.99 n=198
Hemoglobin	0.64	0.69	0.28	0.94
Lymphadenopathy	0.62	0.34	0.94	0.66
Hepatosplenomegaly	0.80	0.98	0.42	0.58
Mediastinal mass	0.93	0.84	0.53	0.32
Year of accrual	0.62	0.93	0.90	0.077
Median height for age	0.90	0.49	0.061	0.65
Median weight for age	0.99	0.92	0.22	0.70
Median height and weight for age	0.89	0.83	0.35	0.79

22), yielding a BCR-ABL fusion and t (4; 11), which juxtaposes MLL to AF4. These translocations define clinicopathological entities that have also been used in risk stratification for treatment purposes. An analysis of the presence or absence of the more common chromosomal translocations was, therefore, undertaken. This revealed significant differences from published Western series.

More than 250 newly diagnosed cases of pre B-ALL were analysed by real time multiplex RT – PCR for the four leukemia specific translocations mentioned above (12)). The frequency of t (12; 21) which is associated with a good prognosis was significantly lower in Indian series (7%) than in the USA (22%) or Europe (23%) ($P<0.005$). In contrast t (1, 19) and t (9; 22), the latter generally having a poor

Table-4 : Comparison of WBCs in Various Series in India and the USA or Europe

Institution	<50,000 per cu mm	>100,000 per cu mm	EFS (4-5yr)
CI (Chennai, India)	65.5%	23.2%	43%
AIIMS (Delhi, India)	68.9%	18.0%	41%
TMH (Mumbai, India)	75.4%	14.6%	60%
UK ALL XI (1990-1997) [15]	77.9%	12.0%	63%
POG AlinC14 and 15 Studies [16]	85%	6.6%	66.6%
ALL-BMF 83 [17]	80.2%	11.3%	64.3%
ALL-BMF 90 [17]	77.7%	12.4%	78.0%
St Jude 1988-91[18]	77.7%	13.8%	67.6%
St Jude 1991-94 [18]	73.3%	14.5%	76.9%
Dana Farber CI Study (USA) [19]	81.7%	10.9%	83%

[15]Eden OB et al; Leukemia 14; 2307-2320, 2000.
[16]Maloney et al; Leukemia 14; 2276-2285, 2000.
[17]Schrappe M et al. Leukemia 14; 2205-2222, 2000.
[18]Pui C-H et al. Leukemia, 14; 2286-2294, 2000.
[19]Silverman LB et al, Blood 97; 1211-1218, 2001.

prognosis, appear to be more commonly seen in the Indian series (7% and 5% respectively) than in western series (< 3.8% and <2.2%).

Immunoglobulin and T cell receptor (TCR) gene rearrangement in childhood ALL (Ref. 9)

Briefly, the molecular rearrangements observed were different from the West in terms of higher incidence of TCR-beta rearrangement, invariable deletion of Cgamma 1 and only monoallelic rearrangement of TCR-delta locus. Further, TCR beta arrangement in B cell precursor ALL was associated with a higher mean age at presentation, lower mean platelet count and a poorer disease free survival (% cumulative survival 0 versus 88.9±10.5, p=0.004)

Incidence, clinical characteristics and early treatment outcome in Indian patients of childhood ALL with ALL-1 gene rearrangement (Ref. 10)

Briefly, in 185 patients with median age of 7 years, the incidence of ALL-1 gene rearrangement was found to be 11.4%, conforming to the Western pattern. It was

associated with significantly high WBC count (p=0.01) and CD10 negativity (p=0.00000001). Complete remission and relapse rates in 98 patients evaluable for response to therapy on a uniform therapy protocol were independent of ALL-1 gene status.

Detection of BCR-ABL transcripts in acute lymphoblastic leukemia in Indian patients (Ref. 11)

A semi-nested cDNA-PCR was employed to detect the presence of BCR-ABL chimeric transcripts in 33 patients of ALL. They were found in 24% of children and 19% adults which is in sharp contrast to the published reports from the West where the presence of BCR-ABL has been reported in only 2-5% children and 35% adults. The significance of these results is that the BCR-ABL fusion transcript which is an indicator of poor prognosis may contribute to chemo-incurability in young Indian patients

Significance of MDR1, MRP1, GST pi and GST mu mRNA expression in Acute Lymphoblastic Leukemia in Indian patients (Ref.13)

Semi-quantitative RT-PCR in 167 patients of ALL showed significantly higher MDR 1 expression with age more than 15 years and higher MRP1, GST pi and GST mu expression with WBC counts more than 100 x 10^9/L. Inability to achieve complete remission was associated with a significantly higher MDRI expression in patients less than 25 years of age.

Significance of expression of MDR1, MRP, BCL2, BAX mRNA and BCL2/BAX ratio in ALL in children and young adults (Ref.14)

Using the cDNA-PCR approach in 57 samples drawn from ALL patients with a median age of 9 years, the MDR1 and MRP mRNA levels did not differ in patients having unfavourable treatment outcome. However, higher BCL2 values and higher BCL2/BAX ratios were associated with unfavourable treatment outcome. Thus the disease free survival was significantly better in patients having very low (less than the 30^{th} percentile) BCL2 mRNA levels and significantly poor for patients having BCL2/BAX ratio above the 80^{th} percentile. Thus BCL2 and BCL2/BAX ratio may significantly influence prognosis in denovo ALL in this age group.

Generally speaking, age, an important prognostic factor in Western series, was not associated with outcome. Age is, however, merely a surrogate marker and probably reflects differences in a number of other factors, such as the specific molecular subtype of leukemia (which tend to be age associated), and perhaps immunological and other factors. It is possible that the lack of an association of age with outcome in India is indicative, at least in part, of differences in the pattern of molecular subtypes.

Further, delayed diagnosis and a higher WBC at presentation probably accounts for at least some of the generally worse outcome in Indian patients.

While general principles learned in Western series provide the present foundation for treatment strategies, differences in the population treated, differences in environment and genetics, differences in tumor biology and in the quality of care received are likely to give rise to differences in the results achieved with the same treatment protocols. Thus, the criteria used for risk stratification in Western studies may not be appropriate for use in Indian patients. In addition, it is likely that the molecular study of tumors, including gene expression profiling of tumors, will provide new insights into genetic and environmental factors that determine development of ALL and into factors which influence the outcome of treatment.

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