Indian Journal of Pathology and Oncology 2024;11(3):270–276

Content available at: https://www.ipinnovative.com/open-access-journals

Indian Journal of Pathology and Oncology

Journal homepage: www.ijpo.co.in

Original Research Article

The immunophenotypic and clinicohematological characteristics of T-ALL cases detected over a three-year period in a tertiary institution

Taniya Sharma[1], Urmila Thiyam[1], Shitalmala Thangjam[1], Rajkumari Banashree[1]*, Irom Anil Singh[1], Kshetrimayum Achouba Singh[1]

[1] *Dept. of Pathology, Jawaharlal Nehru Institute of Medical Sciences, Imphal, Manipur, India*

ARTICLE INFO

Article history:
Received 03-06-2024
Accepted 16-07-2024
Available online 12-09-2024

Keywords:
Flow cytometry
Immunophenotyping
T- cell
Deoxynucleotidyl transferase

ABSTRACT

Background: A malignant tumor of immature T cells, T-cell acute lymphoblastic leukemia/lymphoma is known by this acronym, T-ALL. 12-15% percent of all cases of acute leukemia are T-ALL. According to the 2017 WHO classification, early T-cell precursor acute lymphoblastic leukemia/lymphoma (ETP-ALL) is a unique and uncommon condition. It includes 17–22% of adult T-ALL cases and 12–16.2% of childhood T-ALL cases.

Aim and Objective: To examine the immunophenotypic and clinicohematologic features of T-ALL.

Materials and Methods: A retrospective analysis was conducted on all acute leukemia diagnoses made in the Pathology Department, Jawaharlal Nehru Institute of Medical Sciences, Imphal between June 2020 and June 2023, a period of three years. After completing all required tests, such as a CBC and a bone marrow examination, flow cytometric immunophenotyping was performed either from peripheral blood or the bone marrow aspirate. For immunophenotyping, a 11 color flowcytometer (BECKMAN COULTER) was utilized. Markers for T cell lymphoid lineage included CD3, CD5, CD4, CD7, CD8, and cCd3 while markers for B cell lineage included CD19, CD20, CD22, CD10 and cCD79a. Regarding the myeloid markers, CD117, CD13,CD38, and Myeloperoxidase ; the immaturity markers such as CD 34 and HLA-DR; and the monocytic markers CD33, CD14, CD64 and CD11c were used. ETP-ALL diagnosis was accomplished using specific scoring systems.

Results: Out of the 150 acute leukemia cases that were diagnosed during this time, 15 (10%) were categorized as T-ALL according to WHO guidelines. Median age was calculated as 17 (range: 4–60 years). 11/15 (73.3%) of the cases were male, and 4/15 (26.6%) were female. Of the 4 female cases, 2 were found to have ETP-ALL.

Conclusion: This study was carried out since there is a dearth of data from this region of the nation. Furthermore, because ETP-ALL cases have a bad prognosis, it is important to get a thorough diagnosis.

1. Introduction

Abnormal immature T-cell proliferation results in T-cell acute lymphoblastic lymphoma/leukemia (T-ALL/LBL), a malignant disorder. The thymus and bone marrow are home to aberrant T-cell lymphoblasts that are growing rapidly. It is regarded as a subtype of ALL, the most prevalent form of cancer in children, however it can also strike adults, making for 15% of cases of acute leukemia. 15% of ALL cases in children and 25% of ALL cases in adults are caused by T-ALL.[1] An approximate estimate of the yearly incidence is one per 100,000 individuals. The diagnosis depends on establishing clonality or abnormal pattern of maturity, immature nature of the disease and the T-cell lineage.[2]

* *Corresponding author*.
E-mail address: dr.taniyasharma31@gmail.com (R. Banashree).

https://doi.org/10.18231/j.ijpo.2024.059

Increased N:C ratio, uneven nuclear membranes, and fine nuclear chromatin with little cytoplasm are all seen in T-ALL blasts. Nuclei can be uneven, oval, or circular in shape. Frequent mitosis is indicative of fast proliferation. Therefore, immunophenotyping is necessary to verify the T-cell lineage of blasts and differentiate them, from reactive situations such as lymphocytosis and mature T-cell/peripheral leukemia as differentiating these conditions from T-ALL solely based on morphological basis is not possible. This is where immunohistochemistry (IHC) and flow cytometry (FC) come into play.

Immaturity markers like CD34, HLA-DR and terminal deoxynucleotidyl transferase (TdT) are frequently employed in flow cytometry to distinguish between T-ALL/LBL and mature T-cell leukemia. A surface glycoprotein called CD34 is expressed on a range of progenitor and stem cells during hematopoiesis as well as endothelial cells. However, compared to B lineage blasts, T-cell expression of CD34 is more erratic.[3,4] Antigen-presenting cells are known to express the cell surface receptor HLA-DR. In immature T cells in the thymus, HLA-DR expression is normally nonexistent or low, but it can be increased following activation. TdT, an intra-nuclear DNA polymerase, is typically employed to convey the immature character of T lineage cells. It is only expressed at the most prominent stages of lymphopoiesis.[5]

Another marker that is occasionally utilized is CD117 in the differentiation of ETP-ALL from other T-ALL and myeloid neoplasm. A surface marker protein used to detect hematopoietic stem cells (HSCs), it is also known as stem cell factor receptor (SCFR). Although T-cell precursors exhibit a low degree of expression, mature T-cells do not express it. Reports of T-ALL lacking every immaturity marker have also been made.[1]

According to the 2017 WHO classification, early T-cell precursor acute lymphoblastic leukemia/lymphoma (ETP-ALL) is a unique and uncommon condition characterized by early T-cell markers and stem cell-like characteristics. ETP-ALL is a high-risk subtype of T-ALL that is frequently linked to a dismal prognosis and an increased chance of relapse. It includes 12–16% of pediatric cases and 17–22% of cases of T-ALL in adults. Although it exhibits myeloid or stem cell markers like CD34, CD117, CD13, and CD33, it usually displays absent or <5% CD1a and CD8 expression and CD5 expression in <75%. Rather than having the characteristics of an adult T-cell, ETP-ALL has that of an early hematopoietic stem cell.[6,7]

Compared to other subtypes, it necessitates different therapy and tactics because of its distinct immunophenotypic and genetic characteristics. Thus, the purpose of this study was to investigate the immunophenotype of T-ALL in the Indian population, with a focus on ETP-ALL, particularly in the northeastern region of the nation.

2. Materials and Methods

This is a retrospective study of T-ALL cases that were diagnosed in the Department of Pathology, JNIMS, Imphal, Manipur, over a three-year period (June 2020–June 2023) as a tertiary care facility. 15 T-ALL patients were assessed during this time. All T-ALL patients with >25% blasts in peripheral blood or bone marrow met the inclusion criteria, which also included meeting WHO standards for identifying T-cell lineage, such as strong cCD3 by FC/IHC and total lack of myeloperoxidase (MPO). Ambiguous,mixed T-/myeloid acute leukemia cases were excluded.

2.1. Investigations

A complete blood cell analysis was performed with an ERBA-H560 five-part differential cell analyzer. Giemsa and Leishman stains were used to stain aspirate smears of bone marrow and peripheral blood (PB), and blasts were counted from each of the 500 cells in the bone marrow and 200 cells in the peripheral blood. Additionally, special stains like Sudan black B, periodic acid-Schiff (PAS), and MPO were used.

A Beckman Coulter 11 color flowcytometer was used for flowcytometric immunophenotyping. The markers listed below were employed to create the character:

B cell lineage: CD19, CD20, CD22, CD79a, CD10;

T cell lineage: CD3, cCD3, CD5, CD4, CD8, CD7

Myeloid markers: Myeloperoxidase (MPO), CD13, CD117, CD38, CD33 and CD 15

Immature markers: HLA-DR and CD 34.

Markers of monocytic cells: CD64, CD33, CD14, CD11c.

The flow sample was collected for a maximum of 600 seconds or one lac events, whichever came first. After the time gate was checked, all of the doublets and triplets were taken out. The following gate contained selected viable cells. Based on the side scatter features of CD45 expression, blasts were gated. On the blasts, markers related to lineage were examined. The population's percentage that expresses all marker is recorded, and a positive response is defined as 20% of the population meeting this criterion. 10% of expression was regarded as positive for MPO.

2.2. Statistical analysis

SPSS version 26 and MS EXCEL software were used for statistical analysis. The mean and standard error of the mean were used to express numerical data. Frequency and percentage were used to represent qualitative data. For quantitative data, the student's T-test was used to assess group differences; for qualitative data, the Chi-square test or Fisher's exact T-test was employed. P values below 0.05 were regarded as significant.

3. Results

Hematological profile in clinical practice 17 was the median age (range: 4–60 years). Eleven of the fifteen cases were male, and four were female, or 73.3% and 26.6% of the total. Of the four female cases, two were found to have ETP-ALL. 63% was the mean blast count.

Table 1: Age wise distribution –n (15)

Age Group	Percentage
0-10yrs	2
11-20	9
21-30	0
31-40	2
41-50	0
51-60	2
61-70	0
71-80	0

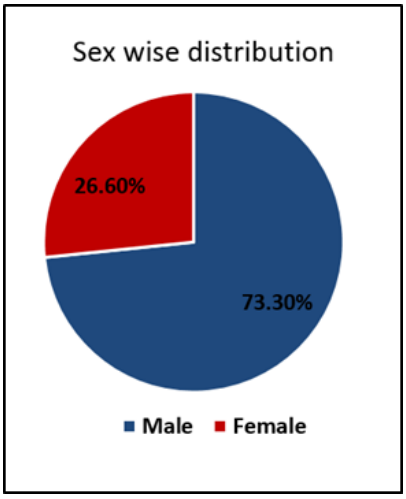

Figure 1: Sex wise distribution

3.1. Immunophenotypic profile

Expression of T lineage markers: 66% of cases showed expression of CD5, 100% of cases showed expression of CD7 and cCD3. The expression of distinct markers is displayed in Table 3. Expression of immaturity markers: CD34 was the most often expressed immaturity marker (expressed in 60% of cases), and 40% of the cases had negative results for all three immaturity markers. Table 4 shows the expression of all immaturity markers (Figure 2).

Expression of aberrant markers: 6/15 cases (40%) had aberrant B cell antigen (CD10/CD79a) expression, while five out of fifteen cases (33.3%) had abnormal expression of one or more myeloid antigens (CD13/CD33/CD117/CD38). CD10 (40%) was the most often expressed aberrant B cell antigen, followed by CD79a in 1/15 (6%) cases. CD13 was the most often expressed aberrant myeloid antigen, appearing in 5/15(33.3%), followed by CD117 in 3/15(20%) and CD33 in 2/15 (13%).

Comparison of the expression of immaturity markers and aberrant antigens between the CD34 positive and negative groups: Two groups were studied (Table 5). We have shown that the lack of CD13 was significantly correlated with CD34 negative T-ALL (P=0.040), and that the expression of aberrant Bcell antigen (CD10, CD79a) was significantly correlated (P=0.028) with CD34 negative groups as well. Figure 2. A dot plot from a T-ALL case depicting the population of interest, or the red bursts. The third plot above demonstrates moderate to brilliant CD45 expression.CD19 is shown as negative in the bottom row and positivity for CD34, CD38, HLA-DR, and CD117. Figure 3 displays negativity for CD3, CD56, CD8, and CD22 and positivity for CD7, CD5, and cCD3.

Acute lymphoblastic leukemia from early T-cell precursors (ETP-ALL): Figure 4 2/15 cases, or 13%, meet the WHO criteria for ETP-ALL. These individuals exhibit myeloid and hematopoietic stem cell markers, such as CD13, CD33, CD34, and CD117 HLA-DR, as depicted in the Figure 3. However, MPO was negative and only expressed CD7 dim CD5 (less than 75% positive cells), while other T lineage markers were negative.

Table 2: Bone marrow findings

Parameter		N=15	Percentage
Cellularity	Hypercellular for age	2	13.3
	Normocellular for age	4	26.6
	Hypocellular for age	9	60
Megakaryocyte	Adequate	4	26.6
	Decrease	11	73.3
	Increase	0	
Erythropoiesis	Reduced	5	33.3
	Adequate or increased	10	66.6
Mean Blast percentage of all nucleated cell		63%	

Table 3: Expression of various markers

S. No.	T-Lineage marker (n=15)	Expression (n,%)
1	SCD3	(2, 13%)
2	cCD3	(15,100%)
3	CD5	(10,66%)
4	CD7	(15,100%)
5	CD4	(2,13%)
6	CD8	(4,26%)
7	Double +	(1,6%)
8	Double-	(10,66%)

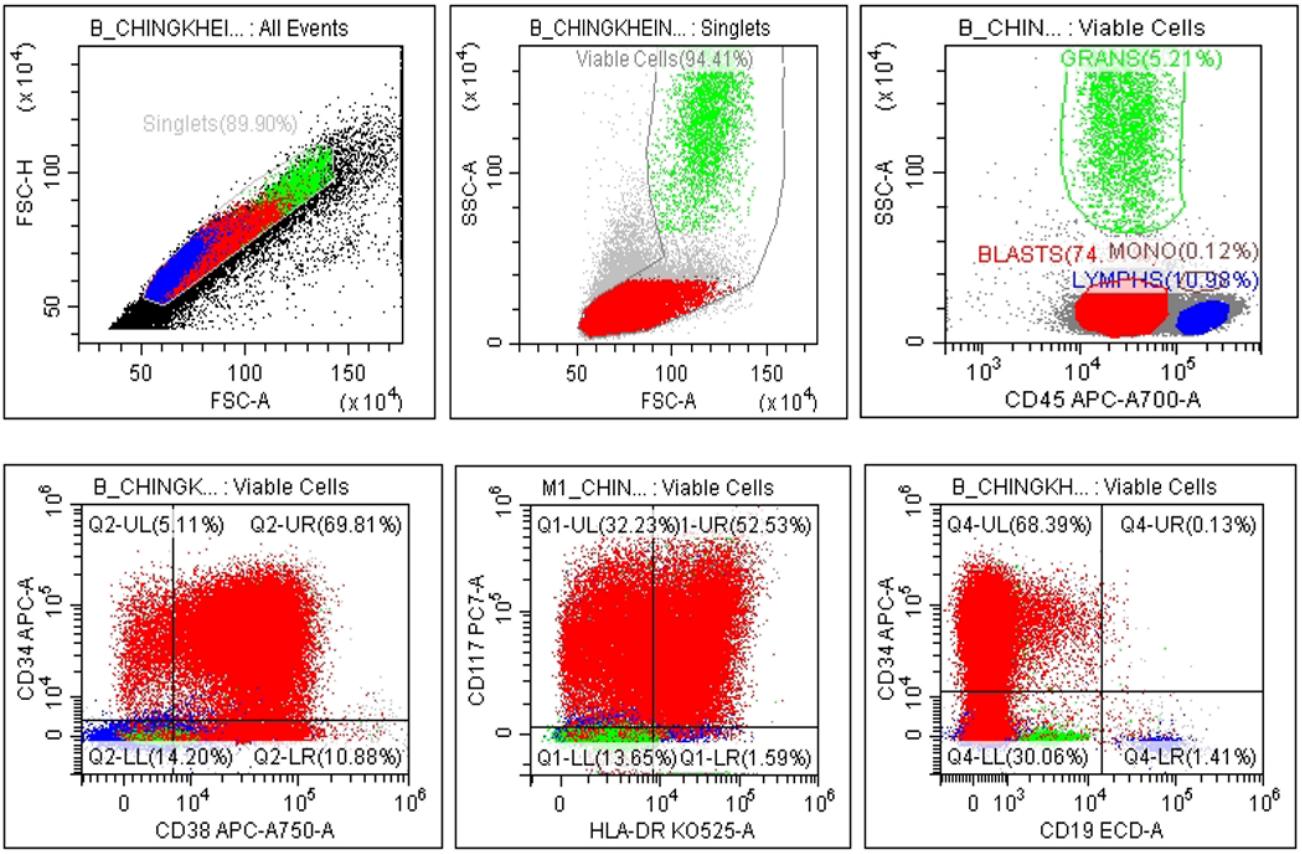

Figure 2: Flow cytometry findings: Immaturity markers: Dot plot from a case of T-ALL showing population of interest i.e. blasts in red. The above third plot shows moderate to bright expression of CD45. The lower row shows positively for CD34, CD38, HLA-DR, CD117 and negativity for CD19

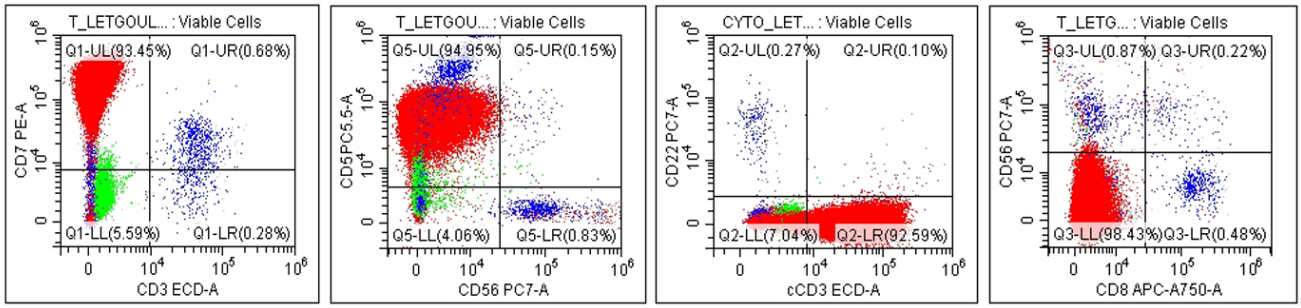

Figure 3: T-cell markers: Here, we can see positivity for CD7, CD5 cCD3 and negativity for CD3, CD56, CD8, CD22

4. Discussion

Clinicopathological and immunophenotypic parameters were examined in 15 instances that were included in the current investigation. The greatest frequency of T-lineage markers expressed by the blasts in our investigation was 100% for cCD3 and CD7, followed by 66% for CD5 (Table 4). These results were consistent with those reported by Gupta et al.[8]

Of the cases in this study, 86.6% had negative HLA-DR, 80% had CD117, and 40% had CD34. The frequency of lack of CD34 ranges from 30.7 to 100% in published literature, while the frequency of absence of HLA-DR ranges from 58.3 to 100%.[4,9–11] In our investigation,40% of the cases tested negative for each of the three immaturity markers, namely CD34–/HLA-DR–/CD117–. We located one study that demonstrates immaturity indicators' negative effects.[1]

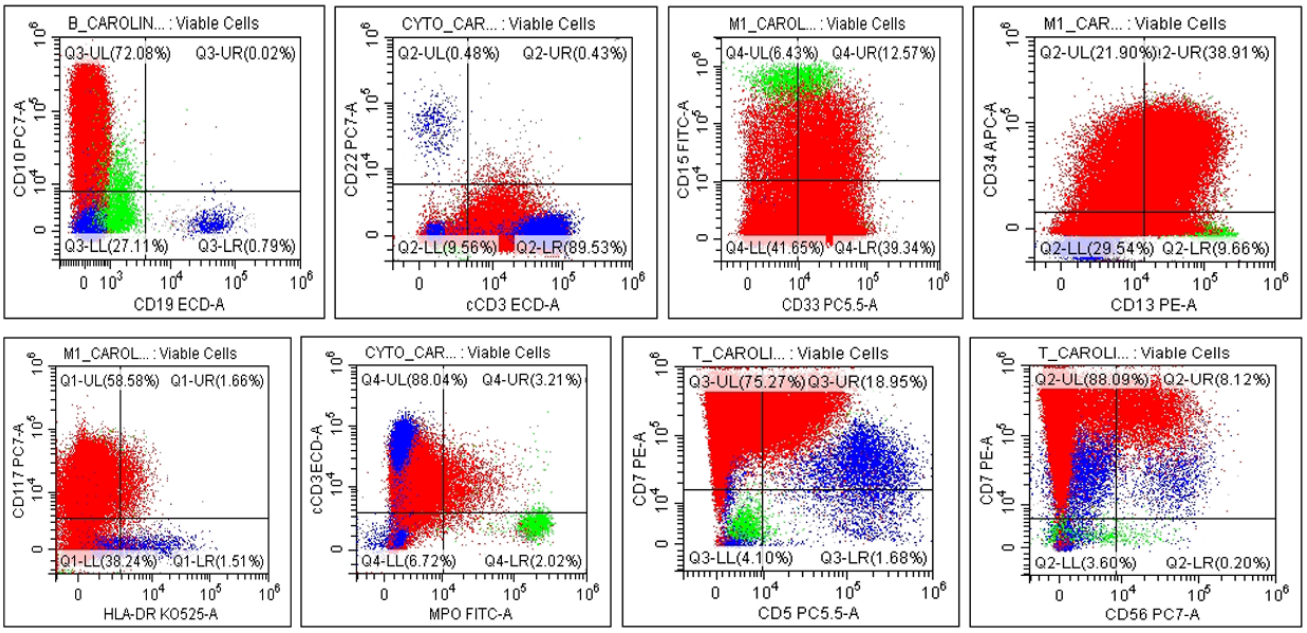

Figure 4: Early T-cell precursor acute lymphoblastic leukemia (ETP-ALL)

Table 4: Expression of all the immaturity markers

S No.	Immaturity markers(n=15)	No. of cases showing expression, (%)
1	CD34	9,(60%)
2	HLADR	2,(13%)
3	CD117	3,(20%)
4	CD34/HLADR/CD117+	2,(13%)
5	CD34+/HLADR+/CD117-	0
6	CD34+/HLADR-/CD117+	1,(6%)
7	CD34-/HLADR+/CD117+	0
8	CD34+/HLADR-/CD117-	7,(46%)
9	CD34-/HLADR+/CD117-	0
10	CD34-/HLADR-/CD117-	6,(40%)
11	CD34-/HLADR-/CD117+	0

Table 5: Comparison between CD34 positive and negative groups

Parameter	CD34+ (9/15)	CD34- (6/15)	p-value
CD13			
Negative	4	6	.04
positive	5	0	
CD33			
Negative	7	6	.48
Positive	2	0	
CD117			
Negative	6	6	.22
positive	3	0	
CD10			
Negative	6	3	.028
Positive	3	3	
CD10, CD79a			
Negative	6	3	.028
Positive	3	3	
HLADR			
Negative	7	6	.48
Positive	2	0	
CD56			
Negative	9	5	1.0
Positive	0	1	

The difference between peripheral T-cell leukemia and T-ALL is determined by the presence of certain markers, such as CD7, CD5, CD1a, and aberrant myeloid/B-cell antigens. We were unable to incorporate the thymic phenotype-suggesting and T-ALL-diagnostic expression of CD1a into our investigation. T-ALL is rarer to have HLA-DR positive than peripheral T-cell leukemia.[12] A single feature cannot diagnose any particular entity hence a methodical approach is required, taking into account factors including the clinical history, the properties of the blasts in the aspirate and biopsy, and most crucially, immunophenotyping.TCR rearrangement and Vβ repertoire are the gold standard, however they are not widely available and also expensive.

The well-known characteristic of aberrancy in leukemias aids in the identification of malignant cells, which are identified by the presence of antigen from a different lineage. Another important factor in differentiating peripheral T-cell leukemias from T-ALL is the presence of B-cell or myeloid antigen.[2] Additionally, they are helpful in prognostication and in differentiating T-ALL cells from normal hematogones while monitoring patients for MRD.[13] Compared to Chen et al.[14] this study reveals abnormal expression of B-cell antigen in 40% of patients. 33.3%

of cases have myeloid aberrancy, which is consistent with earlier research that found it to range between 9 and 50%.[15,16] CD10 was the most often expressed B-cell antigen, followed by CD79a. In contrast, other studies found that the most often expressed aberrant B-cell antigen was CD79a.[17] The majority of research in the literature, found that CD13 was the most often expressed aberrant myeloid antigen, appearing in 5/15(33.3%), followed by CD117 in 3/15(20%) and CD33 in 2/15 (13%) individuals.[8,9,18–21]

It is noteworthy that T-cells without CD34 were strongly linked to the absence of CD13 (P=0.040). Additionally, CD34 negative groups exhibited a significant correlation with the expression of aberrant B cell antigens, such as CD10 and CD79a (P= 0.028). The first leukemic cells of the lymohoblastic lineage often exhibit B-cell antigens, and instances where CD34 expression is lacking are likely due to phenotypically more immature blasts. This association suggests that a lot of cases have very early precursors. Additionally, the lack of CD13 was substantially correlated with CD34-negative T-ALL (P=0.040). Our results were in line with those of Gupta et al.[1] and in opposition to those of Chen et al., who discovered a strong correlation between myeloid antigen expression in the CD34 positive group (36.58%) and the CD34-group (15.38%) (P<0.01).[12] It is thought that the first thymic precursors are dual T and myeloid potential, and they are CD13 positive.[21] Furthermore, the association between it and CD34 negative points to a very early precursor as the possible etiology of many leukemia cases in our study as well. Based on the six and eleven marker combination systems described in the literature,[6] the diagnosis of ETP-ALL was made.

5. Conclusion

T-ALL is an uncommon and severe illness. The lack of follow-up and the small sample size were the limitations. The prognosis for T-ALL and ETP-ALL is not good, with ETP-ALL faring worse. Determining the T-cell lineage aids in prognosis. Because of its rarity and the need for a thorough diagnosis, this study is being done. Bigger follow-up studies are needed for subsequent investigations.

6. Source of Funding

None.

7. Conflict of Interest

None.

References

1. Gupta R, Garg N, Kotru M, Kumar D, Pathak R. Immunophenotypic characteristics of T lineage acute lymphoblastic leukemia: absence of immaturity markers-TdT, CD34 and HLADR is not uncommon. *Am J Blood Res*. 2022;12(1):1–10.
2. Gorczyca W, Weisberger J, Liu Z, Tsang P, Hossein M, Wu CD, et al. An approach to diagnosis of T-cell lymphoproliferative disorders by flow cytometry. *Cytometry*. 2002;50(3):177–90.
3. Faber J, Kantarjian H, Roberts WM, Keating M, Freireich E, Albitar M. Terminal deoxynucleotidyl transferase-negative acute lymphoblastic leukemia. *Arch Pathol Lab Med*. 2000;124(1):92–7.
4. Ross CW, Stoolman LM, Schnitzer B, Schlegelmilch JA, Hanson CA. Immunophenotypic aberrancy in adult acute lymphoblastic leukemia. *Am J Clin Pathol*. 1990;94(5):590–9.
5. Faber J, Kantarjian H, Roberts MW, Keating M, Freireich E, Albitar M. Terminal deoxynucleotidyl Transferase-negative acute lymphoblastic leukemia. *Arch Pathol Lab Med*. 2000;124(1):92–7.
6. Sin CF, Man PM. Early T-Cell Precursor Acute Lymphoblastic Leukemia: Diagnosis, Updates in Molecular Pathogenesis, Management, and Novel Therapies. *Front Oncol*. 2021;11:750789.
7. Mao J, Xue L, Wang H, Zhu Y, Wang J, Zhao L. A New Treatment Strategy for Early T-Cell Precursor Acute Lymphoblastic Leukemia: A Case Report and Literature Review. *Onco Targets Ther*. 2021;14:3795–3802.
8. Gupta N, Pawar R, Banerjee S, Brahma S, Rath A, Shewale S, et al. Spectrum and Immunophenotypic Profile of Acute Leukemia: A Tertiary Center Flow Cytometry Experience. *Mediterr J Hematol Infect Dis*. 2019;11(1):e2019017.
9. Thalhammer-Scherrer R, Mitterbauer G, Simonitsch I, Jaeger U, Lechner K, Schneider B, et al. The immunophenotype of 325 adult acute leukemias: relationship to morphologic and molecular classification and proposal for a minimal screening program highly predictive for lineage discrimination. *Am J Clin Pathol*. 2002;117:380–9.
10. Salem DA, El-Aziz SMA. Flowcytometric immunophenotypic profile of acute leukemia: mansoura experience. *Indian J Hematol Blood Transfus*. 2012;28(2):89–96.
11. Tong H, Wang H, Wang Q, Liu Z, Lu C. Immunophenotypic, cytogenetic and clinical features in Chinese adult acute lymphoblastic leukaemia (ALL) patients. *Ann Acad Med Singap*. 2014;43(3):152–9.
12. Kaleem Z, Crawford E, Pathan MH, Jasper L, Covinsky MA, Johnson LR, et al. Flow cytometric analysis of acute leukemias. Diagnostic utility and critical analysis of data. *Arch Pathol Lab Med*. 2003;127(1):42–8.
13. Tong H, Lu C, Wang Q, Wang H, Wang H. Immunophenotype of Chinese patients with T-lineage acute lymphoblastic leukemia and its association to biological and clinical features. *Acta Haematol*. 2012;127(4):199–204.
14. Chen LJ, Li JY, Wu YJ, Yang H, Qian SX, Wu HX, et al. Immunophenotyping characteristics of T-cell acute lymphoblastic leukemia. *Zhongguo Shi Yan Xue Ye Xue Za Zhi*. 2007;15:692–5.
15. Tong H, Zhang J, Lu C, Liu Z, Zheng Y. Im¬munophenotypic, cytogenetic and clinical fea¬tures of 113 acute lymphoblastic leukaemia patients in China. *Ann Acad Med Singap*. 2010;39(1):49–53.
16. Bhushan B, Chauhan PS, Saluja S, Verma S, Mishra AK, Siddiqui S, et al. Aberrant phenotypes in childhood and adult acute leukemia and its association with adverse prognostic factors and clinical outcome. *Clin Exp Med*. 2010;10(1):33–40.
17. Lahjouji A, Bachir F, Bennani S, Quessar A, Amzazi S. The immunophenotype of adult T acute lymphoblastic leukemia in Morocco. *Exp Oncol*. 2015;37(1):64–9.
18. Gujral S, Badrinath Y, Kumar A, Subramanian PG, Raje G, Jain H, et al. Immunophenotypic profile of acute leukemia: critical analysis and insights gained at a tertiary care center in India. *Cytometry B Clin Cytom*. 2009;76(3):199–205.
19. Iwamoto S, Deguchi T, Ohta H, Kiyokawa N, Tsurusawa M, Yamada T, et al. Flow cytometric analysis of de novo acute lymphoblastic leukemia in childhood: report from the Japanese pediatric leukemia/lymphoma study group. *Int J Hematol*. 2011;94(2):185–92.
20. Sharma M, Sachdeva MU, Varma N, Varma S, Marwaha RK. Characterization of immunophenotypic aberrancies in adult and childhood acute lymphoblastic leukemia: a study from Northern India. *J Cancer Res Ther*. 2016;12(2):620–6.

21. Marks DI, Paietta EM, Moorman AV, Richards SM, Buck G, Dewald G, et al. T-cell acute lymphoblastic leukemia in adults: clinical features, immunophenotype, cytogenetics, and outcome from the large randomized prospective trial (UKALL XII/ECOG 2993). *Blood*. 2009;114(25):5136–45.

Author biography

Taniya Sharma, PG Student https://orcid.org/0009-0001-5704-0754

Urmila Thiyam, Associate Professor

Shitalmala Thangjam, Ex-senior Resident

Rajkumari Banashree, HOD

Irom Anil Singh, Associate Professor

Kshetrimayum Achouba Singh, Professor

Cite this article: Sharma T, Thiyam U, Thangjam S, Banashree R, Singh IA, Singh KA. The immunophenotypic and clinicohematological characteristics of T-ALL cases detected over a three-year period in a tertiary institution. *Indian J Pathol Oncol* 2024;11(3):270-276.