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Original Article

Immunophenotypic characteristics of T lineage acute lymphoblastic leukemia: absence of immaturity markers-TdT, CD34 and HLADR is not uncommon

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Abstract: Introduction: T ALL may show variable morphological features and immunophenotypic analysis for characterisation of immature nature of these cells is needed to establish a diagnosis and distinguish from reactive conditions and mature T cell leukemias. Sometimes immaturity markers-CD34, TdT and HLA DR may not be expressed by blasts. The aim of the present study was to analyse immunophenotype of T ALLs especially with respect to absence of immaturity markers. Methods: Thirty-eight cases of T ALL diagnosed over a period of two and half years were analysed retrospectively with respect to clinical features, haematological features and flow cytometric immunophenotyping for T, B, Myeloid and immaturity markers. Student's *T*-test was used for comparing quantitative data and Chi-square test/Fishers exact *T*-test for qualitative variables. *P* value less than 0.05 was considered significant. Results: The most common T-lineage marker expressed was cCD3 and CD7 which were expressed in 100% cases followed by CD5 in 86.8% cases. The most common immaturity marker expressed was TdT (39.5% cases) followed by CD34 (34.2% cases). Thirteen cases (34.2%) were negative for all three of the immaturity markers i.e. TdT-/CD34-/HLADR. Absence of CD34 was associated with absence of expression of HLA DR ($P<0.05$) and aberrant expression of B lineage markers ($P<0.05$). Conclusion: T-ALL is a rare and aggressive disease. Many cases lack immaturity markers viz, TdT, CD34 and HLADR. In such cases a comprehensive approach taking into account the clinical presentation, cytomorphology and immunophenotyping is diagnostic in experienced hands. Further, molecular studies may be needed to aid diagnosis.

Keywords: T-ALL, flow cytometry, TdT, CD34, HLA-DR, immaturity markers

Introduction

T-acute lymphoblastic leukemia/lymphoma (T-ALL/LBL) is a neoplasm of lymphoblasts committed to the T-cell lineage, involving bone marrow (BM) and blood (in T-ALL) or with primary involvement of the thymus or of nodal or extranodal sites (in T-LBL). A cut-off of >25% blasts in BM is used to define T-ALL. T-ALL is a rare and aggressive disease comprising of 15% and 25% of all childhood and adult acute lymphoblastic leukemias (ALLs). Diagnosis requires establishment of clonality, T-cell lineage and immature nature of the disease [1]. T-cell lineage can be established by positivity for cytoplasmic CD3 (cCD3) which is readily available lineage specific marker [1]. Demonstration of clonality requires flow cytometric assessment of Vβ repertoire or T cell receptor (TCR) rearrangements by molecular methods which is costly and not readily available everywhere [1].

Blasts in T ALL may show variable morphology from large atypical cells with prominent nucleoli to small cells with scant cytoplasm and convoluted nuclei, thus making it difficult to distinguish the disease from reactive conditions with lymphocytosis and peripheral/mature T-cell leukemia on a purely morphological basis. In such cases, the diagnosis can be established by the presence of immaturity markers on blasts using flow cytometry (FC) or immunohistochemistry (IHC).

The commonly used markers used to establish immaturity include Terminal deoxynucleotidyl

transferase (TdT), CD34 and HLA DR. Expression of these markers on T-ALL blasts is variable and incompletely explored, especially in Indian context. TdT, an intra-nuclear DNA polymerase, is expressed only at the earliest recognizable stages of lymphopoiesis and is commonly used to express immaturity of T lineage cells [2]. The reported frequency of TdT in T-ALL range from 11.5% to 100%, it's absence posing a diagnostic pitfall [2, 3]. Zhou et al found that 12% of blasts in T-ALL/LBL may not express Tdt. They further reported that such cases had a significantly higher rate of disease progression, shorter overall survival and aggressive phenotype [4]. Similarly, Kidoguchi et al also reported a case of TdT negative T-ALL with ETP phenotype and aggressive course [5]. The other immaturity markers used include CD34 and HLA-DR. CD34 is a transmembrane glycoprotein expressed on early lympho-hematopoietic stem cells, progenitor cells, and endothelial cells. It is the most frequently used immaturity marker especially in B lineage disease. However the expression of CD34 on T lineage blasts is more inconsistent when compared to B lineage blasts and may be negative in 30-100% of T-ALL cases [2, 3, 6-8]. Similarly, HLADR was also not expressed by blasts in 58.3 to 100% of cases of T ALL [2, 3, 6-8]. To the best of our knowledge, the frequency of T-ALL lacking all the 3 immaturity markers viz TdT, CD34 and HLADR has not been reported.

The present study aimed to study the immunophenotype (IPT) of T-ALL in Indian population along with frequency and significance of T-ALLs lacking the three commonly used immaturity markers; TdT, CD34 and HLADR.

Material and methods

Case selection

This is a retrospective analysis of cases diagnosed as T ALL done over a period of two and a half years in the department of Pathology of a tertiary care hospital in Delhi. Thirty-eight cases diagnosed as T-ALL over these years were evaluated. Ethical approval for the study was taken from the Institutional Ethics Committee for Human Research. Consent of the patient could not be individually taken as this was a retrospective study. Inclusion criteria included patients diagnosed as T-ALL on the basis of >25% blasts in peripheral blood or bone marrow (BM) and WHO criteria of assigning T-cell lineage i.e. strong cCD3 by FC/IHC and completely lacking cytoplasmic myeloperoxidase by FC/IHC/cytochemistry. Cases of mixed T-/myeloid acute leukemia were excluded.

Investigations

Hemogram: Complete blood cell count was done on Beckman coulter LH 500. Hemoglobin, total leucocyte count and platelet count was recorded. Peripheral blood (PB) and bone marrow aspirate (BMA) smears were stained with Wright's stain and definite blasts were counted out of 200 cells in peripheral blood and 500 cells in bone marrow by two individual pathologist and mean of their readings were recorded.

IPT: For Immuno-phenotyping, flow cytometry was utilised (using Beckman coulter cytomics FC 500 flow cytometer equipped with facility for at least 5-color and 2 laser). Standard stain-lyse-wash protocol was employed.

The following markers were used for characterisation: (1) Immaturity markers: CD45/CD34/TdT/HLA-DR; (2) Myeloid markers: MPO/CD117/CD13/CD33/CD64/CD11c/CD15; (3) T lymphoid markers: cCD3/CD3/CD5/CD7/CD2/CD4/CD8/CD1a; (4) B-lymphoid markers: cCD79a/CD10/CD19/CD20.

The tube was then acquired using Cytometry List Mode data (LMD) acquisition and analysis software (CXP Analysis 2.2). The sample was acquired to a maximum of 600 seconds or a total of 1 lac events whichever was earlier. After putting the viability gate, blasts were gated on the basis of CD45 and side scatter characteristics and lineage related markers. Percent gated population expressing CD34, Tdt and HLA-DR was then recorded. A threshold of 20% was used to define a positive reaction of blast cells to CD34 and HLA-DR. For Tdt, 10% level of expression was considered positive.

Statistical analysis

Statistical analysis was performed using MS EXCEL and SPSS software version 26. Numerical data were expressed as mean and standard error of mean. Qualitative data was expressed as frequency and percentage. Differences between groups were evaluated

Table 1. Expression of T-lineage markers

S.no.	T-lineage marker (n=38)	Expression (n, %)
1.	SCD3	13, 34.2%
2.	cCD3	38, 100%
3.	CD5	33, 86.8%
4.	CD7	38, 100%
5.	CD1a	17, 44.57%
6.	CD2	22, 57.9%
7.	CD4	19, 50%
8.	CD8	15, 39.5%

n: total number of cases.

Table 2. Expression of immaturity markers (n=38)

S.no.	Immaturity marker(s) (n=38)	No. of cases showing expression, %
1.	TdT	15, 39.5%
2.	CD34	13, 34.2%
3.	HLADR	5, 13.2%
4.	TdT+/CD34+/HLADR+	2, 5.2%
5.	TdT+/CD34+/HLADR-	2, 5.2%
6.	TdT+/CD34-/HLADR+	0, 0%
7.	TdT-/CD34+/HLADR+	2, 5.2%
8.	Td-/CD34-/HLADR+	1, 2.6%
9.	TdT+/CD34-/HLADR-	11, 28.9%
10.	TdT-/CD34+/HLADR-	7, 18.4%
11.	TdT-/CD34-/HLADR-	13, 34.2%

n: total number of cases.

using Student's *T*-test for quantitative data and Chi-square test/Fishers exact *T*-test for qualitative variables. *P* value less than 0.05 was considered significant.

Results

Clinical-hematological profile

Out of total thirty eight cases diagnosed as T-ALL, there were 27 males and 11 females (male to female ratio of 2.45:1). Twelve cases belonged to age group <10 years with a mean ± SD age of 5±2 years and 26 cases in the age group of >10 years with a mean ± SD age of 25.1±13.6 years. The mean blast count was 66.56%. There were 10 cases with peripheral blood blast count <50% and 28 cases with blast count >50%.

Immunophenotypic profile

Expression of T lineage markers: The most common T-lineage marker expressed was cCD3 and CD7 which were expressed in 100% cases followed by CD5 in 86.8% cases. The expression of various T lineage markers are given in **Table 1**.

*Expression of immaturity markers (**Table 2**):* The most common immaturity marker expressed was TdT (39.5% cases) followed by CD34 (34.2% cases). Thirteen cases (34.2%) were found negative for all three immaturity markers i.e. TdT-/CD34-/HLADR (**Figure 1**).

Aberrant marker expression: A total of 21/38 (55.3%) cases showed aberrant expression of one or more B cell antigen (CD10/CD19/CD79a) while 15/38 (39.5%) cases showed aberrant expression of one or more myeloid antigen (CD13/CD33/CD117/CD15/CD-11c). The most common aberrant B cell antigen expressed was CD79a seen in 31.6% (12/38 cases) followed by CD10 in 28.9% (11/38) and CD19 in 2.6% (1/38 cases). The most common aberrant myeloid antigen expressed was CD33 seen in 23.7% (9/38) followed by CD117 in 15.8% (6/38). CD13, CD15 and CD11c were seen in 10.5% cases each [(4/38), (2/19) and (2/19) cases respectively].

Comparison between CD34 positive and negative group

These two groups were then compared with respect to expression of aberrant antigens and immaturity markers (**Table 3**).

With respect to expression of one or more aberrant B cell antigens, the CD34 negative group showed a significantly higher expression of one or more of the following - CD10, CD19 and CD79a (P=0.029). Also, CD34 negative T-ALL was significantly associated with absence of CD13 and CD33 (P=0.010 and P=0.040 respectively).

With respect to immaturity markers, CD34 negative T-ALL was significantly associated with HLADR negative T-ALL (P=0.038).

Discussion

The present study included thirty-eight cases of T lineage ALL which were studied for their immunophenotypic characteristics especially expression of immaturity markers-CD34, Tdt and HLA-DR. In this study, the most common T-lineage marker expressed by blasts were

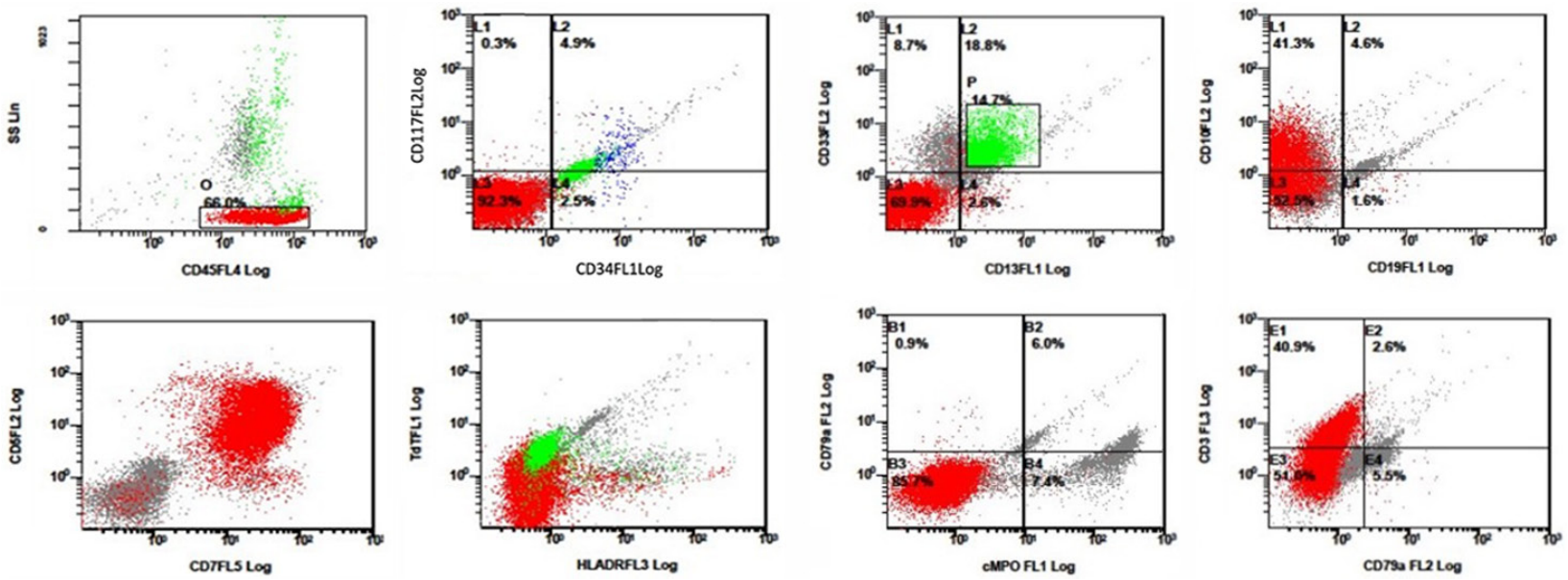

Figure 1. Dot plot from a case of T-ALL showing population of interest ie blasts in red. The above four plots (from left to right) show moderate to bright expression of CD45; negative CD34 and CD117; negative CD13 and CD33; negative CD19 and mild CD10 expression. The below four plots (from left to right) show blasts express bright CD5 and CD7; are negative for Tdt and HLA-DR; negative for cytoplasmic MPO and CD79alpha; and express cytoplasmic CD3.

Table 3. Comparison of T-ALL cases expressing and lacking CD34

Parameter	CD34 Positive (13/38)	*CD34 Negative (25/38)*	*p-value*
CD13			
Negative	9	25	0.010*
Positive	4	0	
CD33			
Negative	7	22	0.040*
Positive	6	3	
CD117			
Negative	04	02	0.685
Positive	46	23	
CD10			
Negative	11	16	0.268
Positive	2	9	
B cell antigen (CD10 + CD19 + CD79a)			
Negative	9	8	0.029*
Positive	4	17	
HLADR			
Negative	24	9	0.038*
Positive	1	4	
TdT			
Negative	9	14	0.429
Positive	4	11	

T-ALL: T-acute lymphoblastic leukemia, *P<0.05 is statistically significant.

cCD3 and CD7 which were expressed in 100% cases followed by CD5 in 86.8% cases (**Table 1**). Our findings are similar to the Indian study by Gupta et al who found cCD3 in 100% (61/61) of T-ALL cases followed by CD7 in 96.7% (59/61) cases and CD5 in 54/61 (88.5%) cases [9].

Most cases of T-ALL were negative for HLADR (86.8%) followed by CD34 (65.8%) and TdT (60.5%). Our results are in accordance with the other studies. The reported frequency of absence of TdT in T-ALL in literature range from 0% to 88.4%, that of CD34 negativity range from 30.7-100% and that of HLADR negativity range from 58.3-100% [2, 3, 6-8]. **Table 4** shows the reported absence of these immaturity markers in T-ALL in literature [2-4, 6-28]. More than one-third cases (34.2%) were negative for all three of the immaturity markers i.e. TdT-/CD34-/HLADR- (**Table 2**). On extensive literature search, we did not find any study highlighting the reasons, frequency and prognostic impact of absence of all three immaturity markers in T-ALL.

TdT-negative T-ALL is derived from an early stage of T precursors lacking TdT expression or from a later stage of precursors with an altered T-cell differentiation [4]. Other methodological reasons for TdT negativity in T-ALL cited in literature are different sensitivities of the antibodies as well as different cut-off levels for the discrimination of positive and negative cases, fluorochrome labelling, varying gates in flow cytometric analysis and different TdT antibodies recognizing distinct TdT epitopes [23]. TdT-negativity is an independent poor prognostic factor in T-ALL and is highly correlated to early thymocyte precursor-ALL (ETP-ALL), higher rate of disease progression and shorter overall survival [5].

The lack of TdT in T-ALL can pose a serious diagnostic challenge especially in cases lacking CD34 and HLADR. Such cases need to be differentiated from reactive lymphocytosis and peripheral/mature T-cell leukemia's. **Table 5** shows the differential features of T-ALL lacking maturity markers, reactive lymphocytosis and peripheral T cell leukemia's [1]. T-ALL usually express CD7, CD5, CD1a and aberrant myeloid/B-cell antigens and the presence of these markers distinguish T-ALL from peripheral T cell leukemia's [12]. Expression of CD1a if present, is suggestive of thymic phenotype and is usually diagnostic of T-ALL [12]. HLADR though an immaturity marker, is rare in T-ALL but common in peripheral T-cell leukemias [12]. However, no single feature is diagnostic of any specific entity. A systematic approach taking into consideration clinical history, cytomorphological features of atypical cells in PB and tissue biopsy if possible along with aberrant immunophenotype is essential for diagnosis. Though establishing clonality by Vβ repertoire and TCR rearrangement is the gold standard, it is not available everywhere and not affordable by many patients.

Aberrant expression is defined as presence of an antigen belonging to some other lineage. This is a well-known phenomenon in leukemias and helps in identification of the malignant

Table 4. Studies with reported absence of immaturity markers (TdT, CD34 and HLADR) in literature [2-4, 6-28]

Serial no. Author	Ethnicity	Number of T-ALL patients studied	TdT negativity (n, %)	CD34 negativity (n, %)	HLADR negativity (n, %)
1. Bradstock et al 1989	Australian	35	NA	NA	30, 85.7
2. Ross et al 1990	Michigan	8	0, 0	NA	5, 62.5
3. Khalidi et al 1999	California	34	3/30, 10	19/28, 67.8	18/26, 69.2
4. Faber et al 2000	USA	26	23, 88.4	NA	NA
5. Thalameer et al 2002	Austria	14	6/14, 42.8	4/13, 30.7	10/13, 76.9
6. Kaleem et al 2003	Washington	21	1, 4.7	20, 95.2	21, 100
7. Vitale et al 2006	Italy	90	NA	59, 66	NA
8. Chen et al 2007	Chinese	NA	NA	N, 69	NA
9. Suggs et al 2007	USA	21	2, 9.5	NA	NA
10. Gujral et al 2009	Indian	351	NA	229/348, 65.8	309/350, 88.2
11. Bachir et al 2009	Morocco	59	13/44, 29.5	34/59, 57.6	35/39, 89.7
12. Dakka et al 2009	Morocco	32	NA	26/31, 84	NA
13. Tong et al 2010	Chinese	16	NA	11, 68.7	12, 75
14. Iwamoto et al 2011	Japanese	231	NA, 15.6	NA, 62.7	NA, 83.3
15. Tong et al 2011	Chinese	24	NA	17, 70.8	18, 75
16. Salem et al 2012	Egypt	13	3, 23	13, 100	13, 100
17. Tong et al 2012	Chinese	46	NA	NA, 71.7	NA, 61.4
18. Zhou et al 2013	USA	59	7, 11.8	NA	NA
19. Tong et al 2014	Chinese	24	NA	NA, 75	NA, 58.3
20. Lahjouji et al 2015	Morocco	40	21, 52.5	17, 42.5	30, 75
21. Sharma et al 2015	Indian	44	16, 36.4	NA	NA
22. Sharma et al 2016	Indian	46	NA	NA, 49	NA
23. Paarikh et al 2017	Indian	555	173, 31.17	NA	NA
24. Jalal et al 2017	Iraq	41	14, 34.1	34, 82.9	25, 60.9
25. Gupta et al 2019	Indian	61	10, 16.4	NA, 55.8	NA, 73.8
26. Rezaei et al 2020	Iran	15	7, 46.6	10, 66.6	2, 86.6

N=Number, T-ALL: T acute lymphoblastic leukemia; NA: Not available; USA: United states of America.

cells/blasts. The presence of myeloid/B-cell antigen is another very useful feature in establishing the diagnosis of T-ALL, distinguishing them from peripheral T-cell leukemias [1]. They also have prognostic impact, are useful in distinguishing T-ALL cells from normal hematogones and in monitoring patients for MRD [22]. **Table 6** shows the comparison of aberrant antigen expression in T-ALL in literature [3, 6, 7, 9, 11-18, 20, 22, 23, 25, 27-30]. In the present study, aberrant expression of one or more B cell antigen was seen in 55.35% cases which is higher than reported by Chen et al (12/140, 8.5%) [14]. Aberrant expression of one or more myeloid antigen was seen in 39.5% cases. Our results are in accordance with literature where it ranges from 9-50% (**Table 6**) [19, 31]. The most common aberrant B cell antigen expressed was CD79a (31.6%) followed by CD10 (28.9%) and CD19 (2.6%). Similar results were shown by Lahjouji et al (**Table 6**) [23]. The most common aberrant myeloid antigen expressed was CD33 (23.7%) followed by CD117 (15.8%). However, CD13 was the most common aberrant myeloid antigen expressed in most of the studies in literature (**Table 6**).

With respect to expression of one or more aberrant B cell antigens, the CD34 negative group showed a significantly higher expression of one or more of the following - CD10, CD19 and CD79a (P=0.029). Phenotypically more immature blasts account for cases where CD34 expression is absent. These B cell antigens are usually expressed in earliest leukemic cells of lymphoblastic lineage and this correlation suggests a very early precursor to be the cause of many cases of leukemia in our study group.

Table 5. Differential features of T-ALL lacking immaturity markers, reactive lymphocytosis and peripheral T cell leukemias [1]

Feature	T-ALL lacking immaturity markers	Reactive lymphocytosis	Peripheral/mature (post thymic) T cell leukemia's involving peripheral blood
Clinical features	Mediastinal mass	History of infections, autoimmune disease, vaccination, drug hypersensitivity etc	Nodal and extranodal involvement (lymph nodes, spleen, liver, intestine, skin etc), erythroderma in sezary syndrome, peripheral cytopenia in T-LGL
Age group	Pediatric usually	Usually young	Young to elderly
Morphology	Monomorphic cell population, Large cells with convoluted/indented nuclei, prominent nucleoli, open chromatin	Pleomorphic cells with wide range of shapes and sizes. Example: Downey cells of infectious mononucleosis where the lymphocytes range in size from small and round, to intermediate with abundant cytoplasm, to frank immunoblasts	Peripheral blood: polymorphic population with increased eosinophils, abnormally large atypical cells, ± rouleax formation, prolymphocytes in T-PLL, granules in LGL, Organs: Effaced architecture, variable cell size, Convoluted nuclear morphology with pale to clear cytoplasm, high endothelial venules, polymorphous cell population including eosiniophils, transmigration of lymphocytes, Cytoplasmic granules in T/NK-LGL, pleomorphic R-S cells in lymph nodes in ALCL, sinusoidal distribution of atypical cells in liver and spleen in Hepatosplenic γδ T-cell lymphoma
Immunophenotyping by flow cytometry			
Side scatter	Normal	Normal	Increased in T/NK-LGL and Adult T-cell leukemia/lymphoma
Forward scatter	Increased	Normal	Regular, Increased or markedly increased in ALCL, Hepatosplenic γδ T-cell lymphoma, Angioimmunoblastic-like T-cell lymphoma
CD45	Dim to moderate	Bright	Bright in T/NK-LGL, Hepatosplenic γδ T-cell lymphoma
Abnormal CD4: CD8 ratio	Majority are CD4+/CD8+ or CD4-/CD8-	Reversed ratio	Occasionally are CD4+/CD8+ or CD4-/CD8-
Aberrant lack or dim expression of one or more of the pan-T antigens	Seen in all cases (sCD3>CD2>CD5>CD7), CD5 and CD7 usually expressed	±	Seen in many of the cases (CD7, CD5, CD2 and sCD3)
NK cell antigens (CD56, CD57 or CD16)	Absent	±	Present in T/NK-LGL, Hepatosplenic γδ T-cell lymphoma
CD30	Absent	Positive in immunoblasts	Positive in ALCL
ALK-1	Absent	Absent	Positive in ALCL
Aberrant expression of myeloid/B-cell antigens	Can be present	Absent	Can be present, CD11b/CD11c can be seen in T/NK-LGL
CD25 and CD5	Absent	Absent	Seen in Adult T-cell leukemia/lymphoma
Dendritic cell markers (CD23 and CD21)	Absent	Absent	Angioimmunoblastic-like T-cell lymphoma
CD1a	Present in thymic phenotype	Absent	Absent
HLADR	Present in few cases	Absent	Present in many cases
Seropositive for HTLV1	Absent	Absent	Adult T-cell leukemia/lymphoma

T-ALL: T acute lymphoblastic leukemia; T-LGL: T large granular cell leukemia; T-PLL: T prolymphocytic leukemia; T/NK-LGL: T/Natural killer cell-large granular cell leukemia; R-S: Reed Sternberg; ALCL: Anaplastic large cell lymphoma.

Table 6. Comparison of aberrant antigen expression in T-ALL in literature [3, 6, 7, 9, 11-18, 20, 22, 23, 25, 27-30]

Study	CD10 (%)	CD19 (%)	CD79a (%)	CD13 (%)	CD33 (%)	CD117 (%)	CD15 (%)	CD11b (%)
1. Ross et al 1990	37.5	NA	NA	NA	NA	NA	NA	NA
2. Khalidi et al 1999	12.9	0	0	14.8	12	NA	12	0
3. Thalameer et al 2002	14.3	0	0	21.4	7.1	0	NA	NA
4. Kaleem et al 2003	19	0	0	0	4.7	0	NA	NA
5. Vitale et al 2006	30	NA	NA	NA	NA	NA	NA	NA
6. Chen et al 2007	19.4	NA	NA	NA	NA	NA	NA	NA
7. Suggs et al 2007	42.8	NA	52.4	14.3	NA	NA	NA	NA
8. Gujral et al 2009	43	2.27	NA	11.5	6.3	3.8	NA	NA
9. Marks et al 2009	NA	NA	NA	51	30	NA	12	NA
10. Bachir et al 2009	21.27	1.7	0	22	18.3	NA	NA	NA
11. Dakka et al 2009	19	NA	NA	NA	NA	NA	NA	NA
12. Iwamoto et al 2011	31.6	0	21.8	20.7	15.2	15.6	NA	NA
13. Salem et al 2012	23	0	0	0	7.7	0	NA	NA
14. Tong et al 2012	26	0	0	21.7	4.3	22.5	NA	NA
15. Mazher et al 2013	NA	2	NA	NA	4	4	NA	NA
16. Lahjouji et al 2015	14	2.5	20.5	42.5	25	NA	NA	NA
17. Sharma et al 2016	NA	6.5	16	25.7	21.7	21.6	NA	NAA
18. Jalal et al 2017	NA	NA	7.3	2.4	2.4	2.4	NA	NA
19. Gupta et al 2019	37.7	3.2	NA	32.7	14.7	19	NA	NA
20. Rezaei et al 2020	26.6	NA	0	0	6.6	13.3	NA	NA

T-ALL: T lymphoblastic leukemia; NA: Not available.

This is a unique finding of our study. Also, CD34 negative T-ALL was significantly associated with absence of CD13 and CD33 (P=0.010 and P=0.040 respectively). Our finding is in contrast to that reported by Chen et al who found significantly a higher positivity rate of myeloid antigen expression in CD34 positive T-ALL (36.58%) than that in CD34 negative T-ALL (15.38%) (P<0.01) [12]. CD13 positive T-ALL is believed to be derived from the earliest thymic precursors, which possess dual T and myeloid potential [29]. And its correlation with CD34 negativity is again suggestive of very early precursor to be the cause of many cases of leukemia in our study group. With respect to immaturity markers, CD34 negative T-ALL was significantly associated with HLADR negative T-ALL (P=0.038). This shows that the expression of both these markers go hand in hand in T-ALL.

To conclude, T-ALL is a rare and aggressive disease. This is the first study reporting the incidence of absence of all three immaturity markers in T ALL. The limitation of the study was its small sample size and lack of follow up. In the absence of, markers of immaturity, a comprehensive approach taking into account the clinical presentation, cytomorphology and immunophenotyping is helpful in experienced hands. However, in difficult cases referral to higher centres for Vβ repertoire and TCR gene rearrangement is required. Larger follow up studies with molecular along with flow cytometry are required for future research.

Disclosure of conflict of interest

None.

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References

[1] Gorczyca W, Weisberger J, Liu Z, Tsang P, Hossein M, Wu CD, Dong H, Wong JY, Tugulea S, Dee S, Melamed MR and Darzynkiewicz Z. An

approach to diagnosis of T-cell lymphoproliferative disorders by flow cytometry. Cytometry 2002; 50: 177-190.
[2] Faber J, Kantarjian H, Roberts WM, Keating M, Freireich E and Albitar M. Terminal deoxynucleotidyl Transferase-negative acute lymphoblastic leukemia. Arch Pathol Lab Med 2000; 124: 92-97.
[3] Ross CW, Stoolman LM, Schnitzer B, Schlegelmilch JA and Hanson CA. Immunophenotypic aberrancy in adult acute lymphoblastic leukemia. Am J Clin Pathol 1990; 94: 590-599.
[4] Zhou Y, Fan X, Routbort M, Cameron Yin C, Singh R, Bueso-Ramos C, Thomas DA, Milton DR, Medeiros LJ and Lin P. Absence of terminal deoxynucleotidyl transferase expression identifies a subset of high-risk adult T-lymphoblastic leukemia/lymphoma. Mod Pathol 2013; 26: 1338-1345.
[5] Kidoguchi K, Yokoo M, Umino A, Aoki S and Kimura S. Terminal deoxynucleotidyl transferase (TdT) negative early T cell precursor acute lymphoblastic leukemia (ETP-ALL) with spontaneous acute kidney injury. Ann Hematol 2020; 99: 885-886.
[6] Thalhammer-Scherrer R, Mitterbauer G, Simonitsch I, Jaeger U, Lechner K, Schneider B, Fonatsch C and Schwarzinger I. 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: 380-389.
[7] Salem DA and Abd El-Aziz SM. Flowcytometric immunophenotypic profile of acute leukemia: mansoura experience. Indian J Hematol Blood Transfus 2012; 28: 89-96.
[8] Tong H, Wang H, Wang Q, Liu Z and Lu C. Immunophenotypic, cytogenetic and clinical features in Chinese adult acute lymphoblastic leukaemia (ALL) patients. Ann Acad Med Singap 2014; 43: 152-159.
[9] Gupta N, Pawar R, Banerjee S, Brahma S, Rath A, Shewale S, Parihar M, Singh M, Arun SR, Krishnan S, Bhatacharyya A, Das A, Kumar J, Bhave S, Radhakrishnan V, Nair R, Chandy M, Arora N and Mishra D. Spectrum and immunophenotypic profile of acute leukemia: a tertiary center flow cytometry experience. Mediterr J Hematol Infect Dis 2019; 11: e2019017.
[10] Bradstock KF, Kirk J, Grimsley PG, Kabral A and Hughes WG. Unusual immunophenotypes in acute leukaemias: incidence and clinical correlations. Br J Haematol 1989; 72: 512-518.
[11] Khalidi HS, Chang KL, Medeiros LJ, Brynes RK, Slovak ML, Murata-Collins JL and Arber DA. Acute lymphoblastic leukemia. Survey of immunophenotype, French-American-British classification, frequency of myeloid antigen expression, and karyotypic abnormalities in 210 pediatric and adult cases. Am J Clin Pathol 1999; 111: 467-476.
[12] Kaleem Z, Crawford E, Pathan MH, Jasper L, Covinsky MA, Johnson LR and White G. Flow cytometric analysis of acute leukemias. Diagnostic utility and critical analysis of data. Arch Pathol Lab Med 2003; 127: 42-48.
[13] Vitale A, Guarini A, Ariola C, Mancini M, Mecucci C, Cuneo A, Pane F, Saglio G, Cimino G, Tafuri A, Meloni G, Fabbiano F, Recchia A, Kropp MG, Krampera M, Cascavilla N, Ferrara F, Romano A, Mazza P, Fozza C, Paoloni F, Vignetti M and Foà R. Adult T-cell acute lymphoblastic leukemia: biologic profile at presentation and correlation with response to induction treatment in patients enrolled in the GIMEMA LAL 0496 protocol. Blood 2006; 107: 473-479.
[14] Chen LJ, Li JY, Wu YJ, Yang H, Qian SX, Wu HX, Lu H, Xu W and Sheng RL. Immunophenotyping characteristics of T-cell acute lymphoblastic leukemia. Zhongguo Shi Yan Xue Ye Xue Za Zhi 2007; 15: 692-695.
[15] Suggs JL, Cruse JM and Lewis RE. Aberrant myeloid marker expression in precursor B-cell and T-cell leukemias. Exp Mol Pathol 2007; 83: 471-473.
[16] Gujral S, Badrinath Y, Kumar A, Subramanian PG, Raje G, Jain H, Pais A, Amre Kadam PS, Banavali SD, Arora B, Kumar P, Hari Menon VG, Kurkure PA, Parikh PM, Mahadik S, Chogule AB, Shinde SC and Nair CN. Immunophenotypic profile of acute leukemia: critical analysis and insights gained at a tertiary care center in India. Cytometry B Clin Cytom 2009; 76: 199-205.
[17] Bachir F, Bennani S, Lahjouji A, Cherkaoui S, Harif M, Khattab M, Nassereddine I, Zafad S and El Aouad R. Characterization of acute lymphoblastic leukemia subtypes in Moroccan children. Int J Pediatr 2009; 2009: 674801.
[18] Dakka N, Bellaoui H, Bouzid N, Khattab M, Bakri Y and Benjouad A. CD10 AND CD34 expression in childhood acute lymphoblastic leukemia in Morocco: clinical relevance and outcome. Pediatr Hematol Oncol 2009; 26: 216-231.
[19] Tong H, Zhang J, Lu C, Liu Z and Zheng Y. Immunophenotypic, cytogenetic and clinical features of 113 acute lymphoblastic leukaemia patients in China. Ann Acad Med Singap 2010; 39: 49-53.
[20] Iwamoto S, Deguchi T, Ohta H, Kiyokawa N, Tsurusawa M, Yamada T, Takase K, Fujimoto J, Hanada R, Hori H, Horibe K and Komada Y. Flow cytometric analysis of de novo acute lym-

phoblastic leukemia in childhood: report from the Japanese pediatric leukemia/lymphoma study group. Int J Hematol 2011; 94: 185-192.
[21] Tong H, Wang Q, Lu C, Liu Z and Hu Y. Immunophenotypic, cytogenetic, and clinical features of 207 cases of childhood acute lymphoblastic leukemia in china. J Pediatr Hematol Oncol 2011; 33: 437-41.
[22] Tong H, Lu C, Wang Q, Wang H and Wang H. Immunophenotype of Chinese patients with T-lineage acute lymphoblastic leukemia and its association to biological and clinical features. Acta Haematol 2012; 127: 199-204.
[23] Lahjouji A, Bachir F, Bennani S, Quessar A and Amzazi S. The immunophenotype of adult T acute lymphoblastic leukemia in Morocco. Exp Oncol 2015; 37: 64-69.
[24] Sharma RK, Puri V, Mutreja D, Kumar S, Chauhan P, Purohit A, Manivannan P, Sazawal S, Mishra P and Saxena R. Applicability of a single 5 color cytoplasmic markers tube as primary panel in routine immunophenotyping of acute leukemia. J Blood Disord Transfus 2015; 6: 309.
[25] Sharma M, Sachdeva MU, Varma N, Varma S and Marwaha RK. Characterization of immunophenotypic aberrancies in adult and childhood acute lymphoblastic leukemia: a study from Northern India. J Cancer Res Ther 2016; 12: 620-626.
[26] Parikh BP, Patel SP, Raiya BN, Vora HH and Jetly DH. Applicability of a single 5 color cytoplasmic markers tube as primary panel for immunophenotyping of acute leukemia: a Gujarat cancer and research institute experience. Indian J Cancer 2016; 53: 349-352.
[27] Jalal SD, Al-Allawi NAS and Al Doski AAS. Immunophenotypic aberrancies in acute lymphoblastic leukemia from 282 Iraqi patients. Int J Lab Hematol 2017; 39: 625-632.
[28] Rezaei MS, Esfandiari N, Refoua S and Shamaei M. Characterization of immunophenotypic aberrancies in adult and childhood acute lymphoblastic leukemia: lessons from regional variation. Iran J Pathol 2020; 15: 1-7.
[29] Marks DI, Paietta EM, Moorman AV, Richards SM, Buck G, DeWald G, Ferrando A, Fielding AK, Goldstone AH, Ketterling RP, Litzow MR, Luger SM, McMillan AK, Mansour MR, Rowe JM, Tallman MS and Lazarus HM. 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: 5136-5145.
[30] Mazher N, Malik N, Imran A, Chughtai O and Chughtai AS. Aberrant expression of CD markers in acute leukemia. Ann Pak Inst Med Sci 2013; 9: 99-102.
[31] Bhushan B, Chauhan PS, Saluja S, Verma S, Mishra AK, Siddiqui S and Kapur S. Aberrant phenotypes in childhood and adult acute leukemia and its association with adverse prognostic factors and clinical outcome. Clin Exp Med 2010; 10: 33-40.