

## THE IMMUNOPHENOTYPE OF ADULT T ACUTE LYMPHOBLASTIC LEUKEMIA IN MOROCCO

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**Background:** There is paucity of detailed studies of adult T cell acute lymphoblastic leukemia (T-ALL) in developing countries reflecting the condition of these patients including clinical and biological features. **Objective:** This study was carried out to analyze the immunophenotypic characteristics of 40 Moroccan patients with T-ALL and its association with biological and clinical features. **Patients and Methods:** Between 2006 and 2009, 130 adult patients diagnosed with acute lymphoblastic leukemia (ALL) were immunophenotyped by 3-color flow cytometry using a panel of monoclonal antibodies. Cases presenting features of a T-lineage phenotype were subjected to detailed analysis including immunophenotypic, clinical and biological parameters. **Results:** Proportion of T-ALL among ALL Moroccan patients was 31.0%. Median age of patients was 28 years. Twenty-nine patients were females and 11 were males. 45.0% of patients (18/40) had features of immature T-ALL stages (pro-T and pre-T ALL), 30.0% (12/40) of CD1a+ cortical T-ALL stage and 25.0% (10/40) had a characteristic phenotype of medullary T-ALL. The frequencies of progenitor cell markers CD10, CD34 and TdT expression were 14.0; 57.5% and 50.0% respectively. The aberrant expression of B lineage associated antigen CD79a were positive in 20.5% of the cases and the aberrant expression of myeloid antigens CD13 and/or CD33 was found in 22 (55.0%) cases. No significant association was encountered between TdT, CD34 or myeloid antigens positivity and high risk features at presentation as age, sex, and white blood cells. However, myeloid antigens (CD13 and/or CD33) was significantly associated with T-cell maturation stages ( $p = 0.009$ ). **Conclusion:** To the best of our knowledge, this is the first report from North Africa of immunophenotypic study on adult T-ALL. Our findings indicate that the proportion of T-ALL among ALL in Morocco is similar to that reported in others Mediterranean countries like France and Italy and that myeloid-associated antigens expression is frequently associated with immature immunophenotype.

**Key Words:** adult T cell acute lymphoblastic leukemia, immunophenotypic analysis, immunological subtypes, myeloid and T-cell antigens.

Acute lymphoblastic leukemia (ALL) represents a biologically and clinically heterogeneous group of diseases characterized by the accumulation of immature lymphoid cells within the bone marrow (BM) and lymphoid tissues. ALL represents about 80% of acute leukemias in childhood, where it is the most frequent malignancy, and accounts for about 20% of adult acute leukemias [1].

Early classification systems for acute leukemias were based only on morphological and cytochemical investigations. Morphology still plays a central role, but current World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues incorporates also immunophenotyping, cytogenetic and molecular characteristics that contribute towards defining biologically and clinically relevant leukemia subsets [2].

Flow cytometric immunophenotyping of ALL is fundamental in the modern diagnosis and treatment of acute leukemia for several reasons. First, the utilization of larger panels of monoclonal antibodies to B-cell, T-cell and myeloid as well as non-lineage-restricted antigens allows characterization of blast cells by line-

age assignment, evaluation of cell maturation, and assessment of phenotypic aberrations. Second, immunophenotyping is also essential for recognizing several subtypes of acute myeloid leukemia (AML) (e.g., minimally differentiated, myeloperoxidase-negative AML and acute megakaryoblastic leukemia) and mixed phenotype acute leukemia. Finally, immunophenotyping is important for monitoring the response to treatment, including detection of minimal residual disease and for identifying markers with prognostic implication [3].

Malignant lymphoid neoplasms involving the presence of neoplastic immature lymphoid cells of T-cell lineage in both the blood and the BM have been designated as precursor T cell acute lymphoblastic leukemia (T-ALL)/lymphoblastic lymphoma (T-LBL) in the recently published WHO classification for hematologic neoplasms. When there is extensive involvement in both the BM and the blood, the preferred term is leukemia. However, if a mass lesion is present with minimal blood and BM involvement, the appropriate term is lymphoma [2].

The most important studies about T-ALL/LBL come from developed countries with regard to their distribution according to the classical intrathymic differentiation compartments, clinical features, description of prognostic factors as well as the outcome. The purpose of this study is to determine, by flow cytometry, the frequency and immunophenotypic features of Moroccan patients with T-ALL. The immunophenotypic findings were correlated to biological and clinical features in comparison with B-lineage ALL.

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**Abbreviations used:** ALL – acute lymphoblastic leukemia; AML – acute myeloid leukemia; BM – bone marrow; ETP – early T-precursor origin; MPO – myeloperoxidase; PB – peripheral blood; T-ALL – T cell acute lymphoblastic leukemia; T-LBL – T cell lymphoblastic lymphoma.

\*\*Institution to which the work should be attributed: National Institute of Hygiene, Rabat 769, Morocco.

## MATERIALS AND METHODS

**Patients.** From January 2006 to December 2009, immunophenotyping was successfully performed in 130 consecutive adults ( $\geq 18$  years) newly diagnosed with ALL (other than L3 subtypes) at the Hematology and Oncology Hospital, Casablanca, Morocco. Cases presenting features of a T-lineage phenotype were subjected to detailed analysis including immunophenotypic, clinical and biological parameters. ALL diagnosis was performed on May-Grunwald — Giemsa smears. BM or peripheral blood (PB) samples were collected, prior to treatment, on ethylenediaminetetraacetic acid (EDTA) tubes. All patients were consented to the routine laboratory and hospital procedures including blood and BM studies. Patients were analyzed for age, gender, morphological FAB subtypes, white blood cells (WBC) count, platelets count and hemoglobin levels, lymphadenopathy, splenomegaly, hepatomegaly and cytogenetic data.

**Flow cytometry.** All EDTA-anticoagulated PB/BM samples were immediately transported to the Cytometry Laboratory of the National Hygiene Institute of Morocco for immunophenotyping. All the samples were evaluated by flow cytometry, using CD45 expression vs side scatter to analyze the blast cell population as described earlier [4]. T-lymphoid antigens included CD1a, CD3, CD4, CD5, CD7 and CD8; B-lymphoid antigens included CD10, CD19, CD22, and cytoplasmic CD79a. Myeloid antigens were myeloperoxidase (MPO), CD13 and CD33. Precursor-cell antigens analyzed were CD34, HLA-DR, and TdT. Positive staining was defined as at least 20% of blast cells showing expression of the marker of interest. T-lineage ALL patients were diagnosed based on the positive expression of CD3 (cytoplasmic and/or surface) and were classified further into three maturational stages that were defined as immature T-ALL stage (pro-T and pre-T ALL): sCD3<sup>-</sup>, cCD3<sup>+</sup>, CD7<sup>+</sup>, CD1a<sup>-</sup>, CD5<sup>+</sup> and/or CD8<sup>+</sup>; cortical T-ALL stage: positive for CD1a (irrespective of other markers), and medullary T-ALL: positive for surface CD3 and negative for CD1a (irrespective of other markers).

**Statistical analysis.** The analysis was done using SPSS 17.0 version. The characteristics of the patients were compared by the exact  $\chi^2$  or Fisher tests. All tests were 2-sided with a  $p$  value of less than 0.05 indicating a statistically significant difference.

## RESULTS AND DISCUSSION

**Immunophenotypic findings.** Of the 130 ALL cases immunophenotyped, 40 (31.0%) cases were classified as having T-ALL and 84 (64.5%) cases were classified as having B-ALL disease. Mixed acute leukemia comprised 6 (4.5%) cases and were dropped from further evaluation. Table 1 summarizes the frequencies of antigens expression found in 40 patients diagnosed with T-ALL in comparison with B-ALL. The cytoplasmic CD3 and CD7 were the only surface T-cell antigens expressed by all cases (100.0%), followed by CD5 (82.0%), surface CD3 (62.5%), CD4 (34.0%)

and CD8 (31.5%). Co-expression of CD4 and CD8 was observed in 21.0% of cases whereas lack of expression of them both was observed in 55.0% of cases. CD10 was found in 5 cases (14.0%) and absent in 31 (86.0%) of 36 cases tested for CD10. CD34 (57.5%), TdT (50%) and HLA-DR positivity (25.0%) were significantly much lower in T-ALL than those in B-ALL (77.5; 84.0 and 100.0%, respectively). The aberrant expression of B-lineage associated antigen CD79a was positive in 20.5% of the cases and the aberrant expression of myeloid antigens CD13 and/or CD33 was found in 22 cases (55.0%), more frequently CD13 than CD33. Indeed, CD13 was observed in 17 (42.5%) patients, whereas CD33 was observed in 10 (25.0%) cases. Both CD13 and CD33 antigens were expressed in 5 (12.5%) cases. No difference was observed in myeloid antigen expression between T-ALL and B-ALL subgroups (55.0% vs 52.5%,  $p = 0.744$ ). According to surface antigens expression by leukemic cells, 45.0% of patients (18/40) had features of immature T-ALL phenotype (pro-T and pre-T ALL), 30.0% (12/40) of cortical T-ALL subtype and 25.0% (10/40) of medullary T-ALL subtype. Early T cell precursor-ALL, a very poor prognosis subgroup defined by its associated distinctive immunophenotype (CD1a<sup>-</sup>, CD8<sup>-</sup>, CD5 weak with stem-cell/myeloid markers) [5, 6], was found in 6 (15.0%) cases.

**Table 1.** Number of patients and frequency (%) cells with individual marker expression in B-ALL and T-ALL

CD	B-ALL (n = 84)	T-ALL (n = 40)	$p$
CD1a	–	12 (31.5)	–
CytCD3	0 (0.0)	40 (100.0)	< 0.001
CD3	0 (0.0)	25 (62.5)	< 0.001
CD4	–	13 (34.0)	–
CD5	–	32 (82.0)	–
CD7	3 (3.5)	40 (100.0)	< 0.001
CD8	–	12 (31.5)	–
CD10	5 (60.5)	5 (14.0)	< 0.001
CD13	29 (36.5)	17 (42.5)	0.507
CD19	84 (100.0)	1 (2.5)	< 0.001
CD22	84 (100.0)	2 (5.5)	< 0.001
CD33	24 (29.0)	10 (25.0)	0.649
CD34	65 (77.5)	23 (57.5)	0.023
CD45	72 (91.0)	36 (97.0)	0.433
CD79a	78 (100.0)	7 (20.5)	< 0.001
TdT	68 (84.0)	19 (50.0)	< 0.001
HLA-DR	84 (100.0)	10 (25.0)	< 0.001
CD4 <sup>+</sup> /CD8 <sup>+</sup>	–	8 (21.0)	–
CD4 <sup>-</sup> /CD8 <sup>-</sup>	–	21 (55.0)	–
CD13 <sup>+</sup> and/or CD33 <sup>+</sup>	42 (52.5)	22 (55.0)	0.744
CD13 <sup>+</sup> / CD33 <sup>+</sup>	10 (12.5)	5 (12.5)	1.0

### Relationship between immunophenotype and disease characteristics

**Clinical characteristics by lineage.** Baseline characteristics of T-ALL patients compared with B-ALL patients are summarized in Table 2. T-ALL and B-ALL significantly differed in their presentation. T-cell disease was more commonly seen in males (72.5% vs 52.5%,  $p = 0.033$ ). T-cell disease was more common in the 18 to 35 years age group (75.0% vs 56.0%,  $p = 0.041$ ). There was an association between T-cell disease and a high WBC with 52.5% of T-ALL patients having a WBC more than 50 000/ $\mu$ l compared with 31.0% of B-cell patients ( $p = 0.021$ ). There was no statistically significant difference between groups in terms of splenomegaly and hepatome-

galy frequencies whereas lymphadenopathy seems to be associated with the T-phenotype rather than with the B-phenotype (84.0% vs 57.0%,  $p = 0.014$ ). Cytogenetic data are available for 96 cases including 71 cases of B-ALL and 25 cases for T-ALL. Normal diploid karyotypes were more frequently in T-lineage ALL patients than in B-lineage ALL patients (55.0% vs 29.0%;  $p = 0.035$ ). The immunophenotype of leukemic blasts from patients with t(9;22) and with t(4;11) were restricted to B-ALL.

**Table 2.** Comparison of clinical and biological features of Moroccan adult patients with B-ALL and T-ALL

Variable	All patients, n (%)	B-ALL, n (%)	T-ALL, n (%)	$p$
	124 (100.0)	84 (67.5)	40 (32.5)	
<b>Age</b>				
Median	31 (18–66)	34 (18–66)	28 (18–66)	
≤ 35	77 (62.0)	47 (56.0)	30 (75.0)	0.041
> 35	47 (38.0)	37 (44.0)	10 (25.0)	
<b>Sex</b>				
male	73 (59.0)	44 (52.5)	29 (72.5)	0.033
female	51 (41.0)	40 (47.5)	11 (27.5)	
<b>FAB</b>				
ALL L1	11 (9.0)	8 (9.5)	3 (7.5)	0.758
ALL L2	98 (79.0)	67 (79.5)	31 (77.5)	
NS	15 (12.0)	9 (11.0)	6 (15.0)	
<b>Clinical features*</b>				
Lymphadenopathy (%)	69 (63.5)	48 (57.0)	21 (84.0)	0.014
Splenomegaly, > 2 cm (%)	43 (40.0)	32 (39.0)	11 (44.0)	0.657
Hepatomegaly, > 2 cm (%)	27 (27.0)	17 (22.5)	10 (40.0)	0.084
<b>Laboratory data</b>				
Leukocyte count Median (range), /μl	27 325 (510–513 050)	21 700 (510–450 000)	50 500 (600–513 050)	
< 50 000/μl	77 (62.0)	58 (69.0)	19 (47.5)	0.021
≥ 50 000/μl	47 (38.0)	26 (31.0)	21 (52.5)	
Hemoglobin level* Median (range), g/dl	7.3 (3.0–15.4)	6.9 (3.0–12.8)	9.2 (4.7–15.4)	
< 10 g/dl	86 (81.0)	72 (89.0)	14 (56.0)	0.001
≥ 10 g/dl	20 (19.0)	9 (11.0)	11 (44.0)	
Platelet count* Median (range), /μl	32 500 (2000–570 000)	30 000 (2000–570 000)	51 000 (10 000–236 000)	
< 100 000/μl	90 (84.0)	71 (86.5)	19 (76.0)	0.220
≥ 100 000/μl	17 (16.0)	11 (13.5)	6 (24.0)	
<b>Cytogenetic**</b>				
Normal	30 (35.5)	19 (29.0)	11 (55.0)	0.035
Abnormal	55 (65.0)	46 (71.0)	9 (45.0)	
Ph	18 (21.0)	18 (27.5)	0 (0.0)	0.008
t(4.11)	4 (4.5)	4 (6.0)	0 (0.0)	0.569
Unsuccessful karyotype	11 (11.5)	6 (8.5)	5 (20.0)	

\*Complete data are available for 107 cases including 82 cases of B-ALL and 25 cases for T-ALL.

\*\*Cytogenetic data are available for 96 cases including 71 cases of B-ALL and 25 cases for T-ALL.

NS – not specified; FAB – French-American-British classification; Ph – Philadelphia chromosome.

**Comparison of clinical characteristics in T-cell maturational stages.** The main clinical characteristics of the 40 patients divided into three immune subtypes according to the pattern of antigens expression are summarized in Table 3. As the number of patients was relatively small and for the purpose of statistic comparison we arbitrarily defined two groups: immature group (pro-T and pre-T ALL stages) and mature group (cortical-T and medullary-T stages). As shown in Table 3, patients in the mature group had a significantly high initial white cell count (more than 50 000/μl) than those in the immature group (59.0% vs 28.0%,

$p = 0.048$ ). In contrast, no significant difference was observed between the two groups in regard to the others initial characteristics such age, sex, platelets, hemoglobin levels, organ (lymph nodes, liver and spleen) enlargement and cytogenetic findings.

**Table 3.** Characteristics of the 40 consecutive patients with T-ALL (n, %)

Variable	Immature (Pro- and pre-T)				$p$
	12 (30.0)	10 (25.0)	22 (55.0)		
<b>Age</b>					
Median	28 (18–66)	23 (18–58)	27 (19–49)	28 (18–66)	
< 35	11 (61.0)	10 (83.5)	9 (90.0)	19 (86.5)	0.140
> 35	7 (39.0)	2 (16.5)	1 (10.0)	3 (13.5)	
<b>Sex</b>					
male	12 (66.5)	9 (75.0)	8 (80.0)	17 (77.5)	0.455
female	6 (33.5)	3 (25.0)	2 (20.0)	5 (22.5)	
<b>FAB</b>					
ALL L1	2 (11.0)	0 (0.0)	1 (10.0)	1 (4.5)	0.332
ALL L2	12 (66.5)	11 (92.0)	8 (80.0)	19 (86.5)	
NS	4 (22.0)	1 (8.5)	1 (10.0)	2 (9.0)	
<b>Clinical features*</b>					
Lymphadenopathy (%)	6 (75.0)	11 (91.5)	4 (80.0)	15 (88.0)	0.570
Splenomegaly, > 2 cm (%)	3 (30.0)	5 (62.5)	3 (43.0)	8 (53.5)	0.414
Hepatomegaly, > 2 cm (%)	4 (44.5)	4 (40.0)	2 (33.5)	6 (37.5)	1.000
Leukocyte count Median (range), /μl	21 900 (600–513 050)	49 800 (1060–496 000)	125 450 (8880–253 000)	79 875 (79 875–496 000)	
< 50 000/μl	13 (72.0)	6 (50.0)	3 (30.0)	9 (41.0)	0.048
≥ 50 000/μl	5 (28.0)	6 (50.0)	7 (70.0)	13 (59.0)	
Hemoglobin level* Median (range), g/dl	7.2 (4.7–14.20)	10.0 (5.0–15.40)	10.15 (7.10–12.50)	10.0 (5.0–15.40)	
< 10 g/dl	7 (78.0)	4 (40.0)	3 (50.0)	7 (44.0)	0.208
≥ 10 g/dl	2 (22.0)	6 (60.0)	3 (50.0)	9 (56.0)	
Platelet count* Median (range), /μl	70 000 (16 000–167 000)	50 000 (10 000–236 000)	50 000 (13 000–131 000)	50 000 (10 000–236 000)	
< 100 000/μl	5 (62.5)	7 (70.0)	6 (85.5)	13 (76.5)	0.640
≥ 100 000/μl	3 (37.5)	3 (30.0)	1 (14.5)	4 (23.5)	
<b>Cytogenetic*</b>					
Normal	5 (55.5)	2 (20.0)	3 (50.0)	5 (31.5)	0.458
Abnormal	3 (33.5)	5 (50.0)	2 (33.5)	7 (44.0)	
Echec	1 (11.0)	3 (30.0)	1 (16.5)	4 (25.0)	

\* Complete data are available for 25 cases.

NS – not specified; FAB – French-American-British classification.

p-value indicated that when comparing the immature group and the mature group. The immature group was consisted of pro-T and pre-T stage, and the mature group was consisted of cortical-T and medullary-T stage.

**Clinical characteristics in relation to TdT, CD34 and myeloid antigens expression in T ALL.** No significant association was encountered between CD34, TdT or myeloid antigens positivity and the presenting clinical and biological features studied (Table 4) apart a statistical significance association between myeloid antigens expression and T-cell maturation stages as 63.5% of the cases expressing myeloid associated markers were of the immature phenotype compared with 22% of the myeloid antigens negative cases ( $p = 0.009$ ).

T-ALL/LBL is an aggressive neoplasm derived from T-cell progenitors. The disease can initially present as lymphoblastic lymphoma involving thymus, nodal, and extranodal sites, and/or as ALL involving primarily PB and BM. This work constitutes the first study to be carried out in Morocco involving characterization of the immunophenotypic profile of cases of adult

T-ALL. Our results on the frequency of T-ALL (31%) subtype was close to the frequency reported by some authors for France (28.5%) [7] and Italy (26.0%) [8] but this frequency was higher to the values found in USA (20.0%) [9] and lower than the percentage reported for Egypt (50%) [10] and India (53.0%) [11]. The reason for the differences in the frequency of T-cell immunophenotype is unclear. According some publications, T-ALL is more commonly associated with low socioeconomic status [12].

When we analyzed the association between clinical and laboratory findings with T-ALL and B-ALL immunophenotypic subtypes of ALL, we could confirm previously published data [13–15] indicating that T-ALL occurs more frequently in males younger than 35 years of age and is usually accompanied by a high WBC count and tumor mass. On the other hand, we confirmed that leukemia of the T-cell lineage is commonly associated with a normal karyotype [16].

Immunophenotyping is essential in the diagnostic workup of T-ALL/LBL. Lymphoblasts express cytoplasmic and/or surface CD3 and other T-lineage makers (CD1a, CD2, CD4, CD5, CD7 or CD8) as well as one or more markers of immaturity, including CD1a, CD10, CD34 and terminal deoxynucleotidyl transferase (TdT). The latter is a unique intranuclear DNA polymerase that

catalyzes the template-independent addition of deoxynucleotides to the 3'-hydroxyl terminus of oligonucleotide primers [17] causing insertion of N regions during immunoglobulin and T-cell receptor (TCR) gene rearrangements at the DJ and VDJ junction sites [18, 19]. This mechanism of junctional diversity is essential to the development of an adult-type immunoglobulin and TCR repertoire [19]. The presence, frequency, and clinical significance of TdT in T-ALL have not been examined extensively. In this study, TdT was expressed in 50.0% of our patients with T-ALL. This expression was lower than that detected in recent studies conducted by Zhou et al. [20] and Patel et al. [21] who detected TdT expression in about 90.0% of T-ALL cases. The reasons for the differences may be attributed to different sensitivities of the antibodies as well as different cut-off levels for the discrimination of positive and negative cases. Others methodological variations in detection of TdT expression like fluorochrome labeling, varying gates in flow cytometric analysis and different TdT antibodies recognizing distinct TdT epitopes must also be taken in consideration when comparing studies on the implications of TdT expression. On the other hand, TdT negative cases should be carefully differentiated from mature T-cell neoplasms particularly in cases that are also negative for HLA-DR and CD34.

**Table 4.** Presenting clinical and biological features in relation to TdT, CD34 and myeloid antigens (CD13 and/or CD33) expression in the studied adult T-ALL patients (n, %)

Variable	TdT+	TdT-	p	CD34+	CD34-	p	MvAg+	MvAg-	p
	19 (50.0)	19 (50.0)		23 (57.5)	17 (42.5)		22 (55.0)	18 (45.0)	
<b>Age</b>									
Median	28 (18–66)	27 (18–50)		31 (18–66)	25.5 (18–58)		30 (19–66)	24 (18–49)	
< 35	14 (73.5)	16 (84.0)	0.693	17 (74.0)	13 (76.5)	1.000	15 (68.0)	15 (83.5)	0.464
> 35	5 (26.5)	3 (16.0)		6 (26.0)	4 (23.5)		7 (32.0)	3 (16.5)	
<b>Sex</b>									
male	13 (68.5)	14 (73.5)	0.721	16 (69.5)	13 (76.5)	0.730	14 (63.5)	15 (83.5)	0.286
female	6 (31.5)	5 (26.5)		7 (30.5)	4 (23.5)		8 (36.5)	3 (16.5)	
<b>FAB</b>									
ALL L1	0 (0.0)	3 (16.0)	0.104	1 (4.5)	2 (12.0)	0.595	3 (13.5)	0 (0.0)	0.265
ALL L2	17 (89.5)	12 (63.0)		19 (82.5)	12 (70.5)		16 (72.5)	15 (85.5)	
NS	2 (10.5)	4 (21.0)		3 (13.0)	3 (17.5)		3 (13.5)	3 (16.5)	
<b>Clinical features*</b>									
Lymphadenopathy (%)	10 (100.0)	8 (72.5)	0.214	10 (77.0)	11 (91.5)	0.593	9 (75.0)	12 (92.5)	0.322
Splenomegaly, > 2 cm (%)	7 (50.0)	4 (40.0)	0.697	6 (37.5)	5 (55.5)	0.434	6 (40.0)	5 (50.0)	0.697
Hepatomegaly, > 2 cm (%)	4 (28.5)	6 (60.0)	0.211	5 (31.5)	5 (55.5)	0.397	3 (23.0)	7 (58.5)	0.111
<b>Leukocyte count</b>									
Median (range), /μl	25 000 (1060–205 000)	106 000 (6070–513 050)		66 000 (600–513 050)	41 000 (1060–496 050)		21 900 (600–513 050)	79 875 (1060–496 000)	
< 50 000/μl	12 (63.0)	9 (47.5)	0.328	11 (48.0)	11 (64.5)	0.289	14 (63.5)	8 (44.5)	0.225
> 50 000/μl	7 (37.0)	10 (52.5)		12 (52.0)	6 (35.5)		8 (36.5)	10 (55.5)	
<b>Hemoglobin level*</b>									
Median (range), g/dl	9.5 (4.7–15.40)	8.85 (7.10–14.20)		7.5 (4.70–14.20)	10.9 (8.00–15.40)		7.6 (4.70–14.20)	10 (5.0–15.40)	
< 10 g/dl	8 (53.5)	4 (50.0)	1.000	12 (70.5)	2 (25.0)	0.032	10 (71.5)	4 (36.5)	0.116
> 10 g/dl	7 (46.5)	4 (50.0)		5 (29.5)	6 (75.0)		4 (28.5)	7 (63.5)	
<b>Platelet count*</b>									
Median (range), /μl	52 000 (12 000–236 000)	35 000 (10 000–131 000)		52 000 (10 000–167 000)	50 000 (12 000–236 000)		52 000 (12 000–167 000)	50 000 (10 000–236 000)	
< 100 000/μl	10 (77.0)	7 (78.0)	1.000	13 (76.5)	5 (71.5)	1.000	10 (77.0)	8 (73.0)	1.000
> 100 000/μl	3 (23.0)	2 (22.0)		4 (23.5)	2 (28.5)		3 (23.0)	3 (27.0)	
<b>Cytogenetic*</b>									
Normal	7 (46.5)	3 (43.0)	0.896	7 (46.5)	3 (30.0)	0.659	5 (38.5)	5 (42.0)	0.923
Abnormal	5 (33.5)	3 (43.0)		5 (33.5)	5 (50.0)		5 (38.5)	5 (42.0)	
Echec	3 (20.0)	1 (14.0)		3 (20.0)	2 (20.0)		3 (23.0)	2 (16.0)	
<b>T cell stage*</b>									
Immature	6 (31.5)	10 (52.5)	0.189	12 (52.0)	6 (35.5)	0.289	14 (63.5)	4 (22.0)	0.009
Mature	13 (68.5)	9 (47.5)		11 (48.0)	11 (64.5)		8 (36.5)	14 (78.0)	

\*Complete data are available for 25 cases.

NS – not specified; FAB – French-American-British classification.

CD7 was always detected in our T-ALL series. Although it is one of the most sensitive T-cell marker, CD7 lacks specificity, as cases of AML or natural killer cell leukemia can express CD7 too [22]. In the current WHO classification system [2], CD3 expression is considered the only T-cell lineage-specific marker and is required for T-cell lineage assignment.

CD10 was detected in 14.0% of the T-ALL cases and CD34 was expressed in 57.5% of cases. The CD10 antigen was originally known as common ALL antigen (cALLA). It was one of the first markers to identify acute leukemia in children. In the hematopoietic system, CD10 regulates stromal dependent B lymphopoiesis. The majority of B-lineage ALL cases expressed CD10. However, expression of CD10 has also been reported in other types of leukemia [23]. CD34 is a human stage-specific hematopoietic differentiation antigen, and expressed in early-undifferentiated hematopoietic stem cells, both in lymphoid and myeloid pathways. In leukemic cells, it remains expressed over several stages of lymphoid and myeloid maturation [24, 25]. In our study, the incidence of CD34 expression appeared to be more frequent on B-ALL (77.5%) than T-ALL (57.5%). This is consistent with previous observations in both children [26–28] and adult series [22, 29], suggesting a more immature hematopoietic cell involvement in B-ALL.

According to the stages of normal thymocyte development that they resemble, we stratified our 40 adult T-ALL cases into three maturational groups. The majority of cases (45.0%) arise from immature compartment (pro-T and pre-T ALL). This finding is in agreement with previous reports [30, 31]. Two recent studies have shown that a subset of T-ALL/LBL cases with an immunophenotype considered being of an early T-precursor origin (ETP) belongs to a very high risk group. The ETP immunophenotype is described as lack of CD1a and CD8, CD5<sup>-</sup>/weak, and expression of one or more myeloid or stem cell-related antigens [5, 6]. Both studies focused on pediatric patients and showed that ETP-positive cases represent 5–13% of T-ALL/LBL patients. In our study, we found that 6 cases (15.0%) met the criteria for early T-cell immunophenotype defined by the following immunoprofile: CD1a<sup>-</sup>, CD8<sup>-</sup>, and CD5<sup>+</sup>/weak with positivity for myeloid (CD13 and/or CD33) or stem cell (CD34) antigens.

Malignant blasts often have an abnormal phenotype that allows distinction from normal immature cells. One of these abnormalities is the occurrence of aberrant phenotypes (or anomalous expression) which is defined by the co-expression of markers usually not present on cells of that particular lineage [32]. The frequency of aberrant expression of the B-cell antigen receptor complex associated protein CD79a in our T-ALL cases was 20.5% which was higher than 5.0% recently reported by Patel et al. [21]. The finding of CD3/CD79a coexpression in ALL raises the question of whether these CD79a positive cases are genuinely of T-cell lineage. Pillozzi et al. [33] performed gene rearrangement studies of the immunoglobulin heavy

chain and TCR gamma genes in a series of CD3<sup>+</sup> ALL with or without CD79a expression detected by immunohistochemistry. All of the cases studied showed TCR gamma gene rearrangement, and none showed solely immunoglobulin heavy chain gene rearrangement, strongly suggesting that CD3<sup>+</sup> cases of ALL are of T-cell lineage, regardless of CD79a expression.

The incidence of aberrant myeloid antigen expression in our study is 55.0%, an incidence much higher than reported previously for adult patients [21, 29, 31] but more close to 51.0% reported by Marks et al. [34]. Disparities in flow cytometric technology immunophenotyping could explain this different finding. The frequency of myeloid coexpression was comparable in B and in T-ALL (52.5% vs 55.0%,  $p = 0.744$ ). This was different from earlier reports that My antigen was significantly associated with T cell ALL cases [35]. The role of myeloid associated antigens in the prediction of clinical outcome is still controversial. While the few early adult ALL studies had shown an inferior outcome for My<sup>+</sup> ALL patients [36–38] others have found similar outcomes [31, 35].

In conclusion, our findings indicate, first: that the overall immunoprofile of our T-ALL is generally similar to that described in small case series reports [39–41], second: the proportion of T-ALL among ALL in Morocco is similar to that reported in others Mediterranean countries like France and Italy. Third, the expression of myeloid-associated antigens is associated with immature immunophenotype of blast cells.

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