IMMUNOPHENOTYPING FEATURES OF TUNISIAN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

PROFIL IMMUNOPHENOTYPIQUE DE LA LEUCEMIE AIGUE LYMPHOBLASTIQUE CHEZ L'ADULTE EN TUNISIE

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Abstract

While acute lymphoblastic leukemia (ALL) represents up to 80% of childhood leukemia, it constitutes only about 20% of adult acute leukemia. We held this study to investigate the immunophenotypic profiles of adult ALL. From January 2006 to January 2020, a total of 261 adult patients (>18 years old) newly diagnosed with ALL have been enrolled.
There were 61.7% patients with B-ALL. Pre-B ALL and cortical T-ALL were the most common subtypes. HLA-DR, CD34, CD38 and CD10 were significantly more expressed in B-blasts than T-blasts. Aberrant phenotypes were found in 16.5% of cases. CD33 was the most prevalent myeloid marker in both ALL subtypes. CD117 was only seen in T lineage ALL. CD34 was significantly more present in ALL with aberrant myeloid antigens. Aberrant B and T lymphoid antigens were present in 2% of T-ALL cases and 2.5% of B-ALL, respectively. In our study, the frequency of aberrant phenotypes in adult ALL is rather closer to the lower limit of the reported spectrum.

Key - words: Acute lymphoblastic leukemia; Adults; Immunophenotype; Aberrant phenotype.

Résumé

Bien que la leucémie aigue lymphoblastique (LAL) représente 80% des leucémies aigues de l'enfant, elle constitue uniquement 20% des leucémies de l'adulte. Nous avons mené ce travail afin d'évaluer le profil antigénique des lymphoblastes chez l'adulte.
Entre Janvier 2006 et Janvier 2020, 261 patients âgés de >18 ans ayant une LAL ont été collectés.
Au total, 61,7% des patients avaient une LAL-B. La LAL Pré-B et la LAL-T corticale étaient les sous-types les plus fréquents. HLA-DR, CD34, CD38 et CD10 étaient plus exprimés dans les blastes-B. Un marqueur aberrant (Mab) a été trouvé dans 16,5% des cas. CD33 était le Mab le plus prévalent. CD117 était exprimé exclusivement dans les LAL-T. L'expression du CD34 était plus fréquente dans les LAL exprimant un MAb. Un MAb type B ou T était respectivement trouvé dans 2% des LAL-T et 2,5% des LAL-B.
Dans notre étude, la fréquence des MAb dans la LAL de l'adulte est plutôt proche de la limite inférieure de l'intervalle rapporté dans la littérature.

Mots-clés : Leucémie aigue lymphoblastique ; Adulte ; Immunophénotypage ; Phénotype aberrant.

ملخص

بالرغم من أن ابيضاض الدم الليمفاوي الحاد (ALL) يمثل 80٪ من حالات ابيضاض الدم الحاد لدى الأطفال، إلا أنه يشكل 20٪ فقط من سرطان الدم لدى البالغين. قمنا بهذا العمل لتقييم ملف التنميط المناعي لهته الخلايا اللمفاوية البانية لدى البالغين. بين جانفي 2006 و جانفي 2020، تم تقييم 261 مريضًا تزيد أعمارهم عن 18 عامًا لديهم ALL.
تم جمع 61.7 ٪ من المرضى لديهم ALL-B. كان كل من Pre-B ALL و T-ALL القشري أكثر الأنواع الفرعية شيوعًا. تم التعبير عن HLA-DR و CD34 و CD38 و CD10 بشكل أكبر في blast-B. تم العثور على علامة شاذة (Mab) في 16.5٪ من الحالات. كان CD33 هو Mab الأكثر انتشارًا. تم التعبير عن CD117 حصريًا في LAL-T. كان تعبير CD34 أكثر شيوعًا في ALL معربًا عن MAb. تم العثور على النوع MAb B أو T MAb في 2٪ من LAL-T و 2.5٪ من LAL-B ، على التوالي. في دراستنا ، فإن تواتر MAb لدى البالغين و لديهم ALL; قريب إلى حد ما من الطرف الأدنى من النطاق المذكور في الأدبيات.

الكلمات المفاتيح : ابيضاض الدم الليمفاوي الحاد ; الكبار ; التنميط المناعي ;النمط الظاهري الشاذ.

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a clinically and biologically heterogeneous hematologic malignancy resulting from clonal proliferation of immature lymphoid cells blocked at specific point of the multiple stages of normal lymphocyte differentiation [1]. Pursuant to current World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues [2], ALL diagnosis and classification are established on the basis of multiparametric approach including cytomorphologic, immunophenotypic, cytogenetic and molecular characteristics. While ALL represents up to 80% of childhood leukemia [3], it is less frequent in the elderly and constitutes only about 20% of all adult acute leukemias [4]. Consequently, knowledge concerning this disease in adults is still low-compared to children. Despite the tremendous progress in diagnosis and treatment methods during last decades, the prognosis of adult ALL still remains not satisfying regarding complete remission, overall survival and remission duration [5].Immunophenotyping study is a fundamental tool for diagnosis, classification, monitoring and prognosis evaluation of ALL [2]. Thus, a better assessment of immunophenotypic features and its implication in therapeutic management and monitoring for minimal residual disease could improve the outcome of adult patients with ALL. Immunophenotypic profiles of Tunisian adult patients with ALL are investigated in this study.

METHODS

From January 2006 to January 2020, a total of 261 consecutive adult patients (>18 years old) newly diagnosed with ALL at the biological hematology department of Aziza Othmana Hospital were enrolled in this study. Immunophenotyping was performed on EDTA- anticoagulated bone marrow samples by flow cytometry XL-MCL Beckman Coulter® (3 colors) for samples received between January 2006 and September 2011 and FC500 Coulter® (5 colors) for the remaining period of study. The panel of monoclonal antibodies was provided by Beckman Coulter® (CD45, HLA DR, CD34, CD38, CD19, CD10,CD20, CD3, CD2, CD7, CD56, CD4, CD8, CD13, CD33, CD117, CD65, CD15). cCD3, cMPO and cCD79a were performed in intracytplasmic staining using KIT INTRAPREP Coulter®.

A total of 20000 events were required for flow cytometry acquisition. Gating strategy was performed on the CD45-SSC scattergram. European Group for the Immunological Characterization of Leukemias (EGIL) classification was used [6].
Statistical analysis was done using SPSS 20.0 version. Chi-square test was used to compare qualitative variables. The association of qualitative and quantitative variables was made by student T test. *p* value of less than 0.05 indicates a statistically significant difference.

RESULTS

General characteristics:

The sex ratio was 0.41. The mean age was 39.2 years old [19-84]. Ten percent of patients collected were aged more than 60 years old.
One hundred sixty one (61.7%) patients were classified as having B-ALL and 100 (38.3%) remaining patients had T-ALL. As shown in table I, patients with B-ALL were significantly older and T-ALL patients were predominantly male.

Table I : Demographic characteristics according to ALL subtypes

		B-ALL (161)	T-ALL (100)	p Value
Median age (years)		42	35	<0.01
Gender	Female	85 (52.8%)	24 (24%)	
	male	76 (47.2%)	76 (76%)	<0.01

Abbreviations: B-ALL, B acute lymphoblastic leukemia ; T-ALL, T acute lymphoblastic leukemia

Immunophenotyping profile:

Comparison of the rate of expression of HLA-DR, CD34, CD38 and CD10 between B and T blasts is summarized in Table II.

Table II: Comparison of HLA-DR, C34, CD38 and CD10 expression in patients with T and B-ALL.

	B-ALL (161)	T-ALL (100)	P Value
HLA-DR	155 (96.3%)	14 (14%)	<0.001
CD34	119 (74%)	31 (31%)	<0.001
CD38	114 (70.8%)	88 (88%)	0.01
CD10	144 (89.4%)	26 (26%)	<0.001

Abbreviations: B-ALL, B acute lymphoblastic leukemia;T-ALL, T acute lymphoblastic leukemia

The latter markers were significantly more present in B-blasts than T- blasts. Frequencies of expression of T antigens in T blasts are illustrated in table III.

Table III: Frequencies of expression of T cell markers in T-ALL

	T-ALL (n=100)	
	Number	Percentage
CD3	12	12%
CD7	95	95%
CD2	53	53%
CD5	58	58%
CD4+/CD8+	22	22%
CD4-/CD8-	48	48%

Abbreviation: T-ALL, T acute lymphoblastic leukemia

Regarding B-ALL, CD19 and cCD79a were expressed in all cases. CD20 were found in 20.5% of B-Blasts.

ALL subclassification:

Immunological subsets of both T-ALL and B-All according to EGIL classification are illustrated in table IV.

Table IV: Immunophenotypic EGIL classification of T-ALL and B-ALL

T-ALL		B-ALL	
Subsets	Prevalence (%)	Subsets	Prevalence (%)
T-I (pro-T) ALL	16 (16)	B-I (pro-B) ALL	10 (6.2)
T-II (Pre-T) ALL	35 (35)	B-II (common) ALL	64 (39.8)
T-III (cortical) ALL	42 (42)	B-III (pre-B) ALL	86 (53.4)
T-IV (mature) ALL	7 (7)	B-IV (mature) ALL	1(0.6)

Abbreviations: B-ALL, B acute lymphoblastic leukemia ; T-ALL, T acute lymphoblastic leukemia

Aberrant phenotypes

Aberrant phenotypes were found in 43 (16.5%) patients: 27 (16.8%) patients with B-ALL and 16 (16%) patients with T-ALL.

Aberrant expressions observed are represented in table V.

Table V: Prevalence of aberrant antigens expression in B-ALL and T-ALL

	B-ALL (n=161)	T-ALL (n=100)	P value
Myeloid marker	24 (14.9%)	14 (14%)	0.84
CD33	9 (5.6%)	9 (9%)	
CD13	8 (5%)	4 (4%)	
CD117	0 (0%)	6 (6%)	
CD65	5 (3.1%)	1 (1%)	
CD15	8 (5%)	0 (0%)	
Aberrant B / T antigens expression	4 (2.5%)	2 (2%)	-
Co-expression of myeloid and B/T antigens	1 (0.6%)	0 (0%)	

Abbreviations: B-ALL, B acute lymphoblastic leukemia; T-ALL, T acute lymphoblastic leukemia

Myeloid aberrant expression (MyAg) in B-ALL and in T-ALL was comparable. CD33 was the most prevalent myeloid marker in both ALL subtypes. CD34 was significantly more present in ALL with MyAg (MyAg+ ALL) compared to those without MyAg (MyAg- ALL) (73.7% vs 26.4%, p=0.03).
Regarding T-ALL subtypes, MyAg was seen in immature T-ALL: 5 cases with T-I (pro-T) and 9 cases with TII (pre-T) ALL. Frequencies of MyAg according to B-ALL subtypes were as follow: 4 in pro-B ALL, 6 in commune B-ALL and 14 pre-B ALL.

DISCUSSION

In this present study, we displayed a detailed presentation of immunophenotyping from 261 Tunisian adults with newly diagnosed ALL. Frequencies of expression of several markers including aberrancies were assessed.

General characteristics:

B-All was more frequent than T-ALL. Patients with B-ALL were significantly older and T-ALL affected predominantly male patients. These conclusions were in line with other studies from literature [7,10].

Immunophenotyping profile:

Flow cytometry play a key role in the diagnostic approach of ALL. It allows to confirm the ALL assignment to T or B lineage according to WHO criteria [11].
Comparing B-ALL patients to those with T-ALL, we confirmed previous observations stipulating that B- blasts have a higher expression of HLA-DR and CD34 in both children and adults ALL [7,9,12,13]. Also, in our study, CD38 was highly expressed in both B and T blast cells but with a significantly higher frequency in T lymphoblasts. While a statistical significance was not achieved, similar result was observed in ALL of childhood [14]. It should be noted that high level of CD38 expression is reported to be linked to favorable overall survival in adult patients with ALL [15]. In our study, the impact on prognosis was not evaluated.
In addition, CD10 which is associated with favorable prognosis in childhood B-ALL [16], was also significantly more present in B-blasts than T-blasts in our series. This latter result was also reported in several series [7,10].
Regarding surface T cell antigens, CD3 was present in 12% of T-lymphoblasts. Lahjouji et al and Tong et al reported a clearly higher expression with frequency of 62% and 54.2 %, respectively [7,9]. CD7 was almost expressed by all cases of T-ALL in agreement with results observed in previous studies [7,9,10]. Although CD7 is the most sensitive marker in T-ALL of both adulthood and childhood, it should be recalled that it is not specific for T-lineage ALL [17]. Concerning CD2, it was expressed in half of T-ALL cases. Similar rates of expression in adults were reported in literature [9,18]. Fifty eight percent of T-ALL cases expressed CD5 in our series. In fact, CD5 seems to be the second most frequent surface T cell antigen [7].
T- Blasts co-expressed CD4 and CD8 in 22 (22%) of our cases. This dual CD4/CD8 positivity has been reported to be associated with a favorable outcome [17]. In our study, we couldn't study its impact on prognosis as data concerning patients outcome were not studied. On the other hand, almost half of T cell blasts did express neither CD4 nor CD8. This frequency is consistent with finding from Moroccan series [7]. This aberrant dual negativity was reported to be more frequent in adult T-ALL compared to pediatric T-ALL which implies that adult T ALL are more immature [13].
On the topic of B cell antigens, CD19 and CD79a were expressed in all B-ALL of our series. In fact, these latter specific B cell markers were the B lineage assignment markers in all cases of B-ALL in different series [7,9,19,20]. CD19 negative B-ALL are extremely rare and make the immunophenotypic diagnosis challenging [21].
CD20 was present in 20.5% of our patients with B-ALL. In literature, CD20 has been reported to be present in approximately 50 to 60% of B-ALL [22,23]. Studies that focused on comparing B-ALL features between adults and children found that the expression of CD20 seems to be higher in children than adults [24,25]. It should be noted that some reports found a worse impact on treatment outcome linked to CD20 expression [25,26].
CD10 was highly expressed in our cases at a frequency of 89.4%. In the series of Jalel et al [19], CD10 was the most frequent expressed marker (80%). CD10 negative B-ALL has been correlated to MLL rearrangement [27].

ALL subclassification:

Prevalence of each subtype varies in available studies. This variability among reports could be

explained, at least partially, by the heterogeneity in sub-classification criteria.

TIII (cortical) and pre-B ALL were the most common subsets of ALL in our series. In several earlier reports, pre-T ALL and commune B ALL were the most common subtypes [9,12,18,20,28]. In 130 Moroccan adults with T-ALL, mature T-ALL was the most frequent subset [7]. In other Iranian and Chinese series and in accordance with our result, cortical T-ALL and common B-ALL were the most common subtypes [9,12]. Curiously, in a Tunisian study of 80 adult and pediatric ALL cases, TIII (cortical) and pre-B ALL were also the most frequent subtypes [8]. Thus, it seems probable that these latter subsets represent the most frequent classes of ALL in our region. Importantly, the predominance of pre-B all in our population could be explained by the low specificity of cytoplasmic chain μ. In fact, it is probable that the detection of cytoplasmic chain μ, which allows shifting B-ALL from the common B-ALL subtype to pre-B ALL group, is overestimated because of a non specific fixation, in addition to the lack of consensus regarding the threshold of positivity. Thus, improving the specificity of our method would probably move common B-ALL as the most frequent B-ALL subtype and thus join the results of the majority of trials.

Aberrant phenotypes

Aberrant phenotypes, which are defined by the co-expression of markers that normally did not belong to that particular lineage, occur in adult ALL with a frequency that differs among studies. This variability in frequencies of aberrant phenotypes expression could be explained by the diversity of flow cytometry instruments, methods and reagents, differences in binding characteristics of monoclonal antibodies, the relatively small number of adult ALL in series, differences in defining antigen positivity and the possibility of misdiagnosis of mixed phenotype acute leukemia in the absence of standardized criteria for aberrant expression [9,29].

We reported a total rate of aberrant expression of 16.5% in our series. In preceding studies, the prevalence of aberrant phenotypes in ALL ranges from 10% to 86.9% [19,30].

MyAg constitutes the most prevalent immunophenotypic aberrancy [12]. This latter result was also supported by our study.

We reported a frequency of MyAg of 16.8% in B-ALL and 16% in T-ALL.

In literature, the expression of MyAg varies from 5% to 86% [7,19,31].

Expression of myeloid antigen in T-lineage and B-lineage ALL was comparable in our study. This latter result is in line with conclusions from Lahjouji et al and Sharma et al trials [7,12]. Nevertheless, Yenerel et al noted a significantly higher frequency of MyAg in adult B-ALL comparing to T-ALL [32].

In our series, CD33 was the most commonly aberrant myeloid marker identified in both B-ALL and T-ALL at a frequency of 6.9% followed by CD13 (4.6%). Sharma et al reported the same result [12]. Expression of CD33 and CD13 is known to be frequent in ALL cases and the frequencies of expression vary among studies ranging from 4% to 45% and 6 % to 55%, respectively [12,18]. Thus, expression of these two latter markers seems to be less common in our patients compared to previous series. CD13 was the most frequent aberrant myeloid marker in the majority of studies [9,19,33]. In a large study of ALL in adults, lymphoblasts express typically CD33 and/ or CD13 in pre-B ALL [34]. We insist again on the wide variability in the frequencies of markers expression among authors due to the above-mentioned factors, essentially the absence of a standardized approach for interpretation.

On the other hand, CD117 was less frequently expressed in our cases in line with data from literature [35]. Moreover, aberrant expression of CD117 was observed only in T- lineage blasts. Tong et al reported similar results as CD117 was only seen in patients with T-ALL at a higher frequency of 30% [9]. In a recent report from Iran, authors consolidate this conclusion and found a statistically significant association of aberrant CD117 expression with T-ALL [13].

Regarding carbohydrates antigens, CD65 was found in 6 (2.3%) ALL patients and CD 15 was noted in 8 (5%) patients with B-ALL. Interestingly, in a study enrolling 56 adult patients with ALL, CD65 and CD15 were the most common aberrant antigens found in 12.2% of cases [18]. CD15 was the second frequent aberrant myeloid marker (15.1%) in a Chinese study of 110 adult patients with ALL [9]. Marks and al reported a frequency of CD65 and CD15 expression in adult T-ALL at 4% and 12%, respectively [10]. Analysis from a large study of adult ALL revealed that lymphoblasts express typically CD65 and CD15 in pro B-ALL [34]. Again, we recall the variability in frequencies of expression reported by available studies and we note that the prevalence of CD65 and CD15 in

ALL remains unclear and probably underestimated as this two markers are not always explored in reports. Consequently, standardization of panels of monoclonal antibodies adopted seems necessary in order to uniform the immunophenotypic profiles of ALL and thus permit comparison between studies. Consistently with data from literature [9,12,31], CD34 showed a statistically significant association with MyAg in ALL. Therefore, the fact that CD34 is normally found in hematopoietic stem cells and thus highly expressed by immature lymphoblasts could explain the high prevalence of MyAg in immature ALL [7].

All myeloid aberrancies in our T-ALL cases were seen in immature subtypes (pro and pre-T). In fact, MyAg is correlated to T-cell maturation [7,36] . The more T-ALL is immature the more myeloid antigens are expressed.

Regarding B-ALL, none of B-IV subtype ALL showed an expression of myeloid antigen while MyAg was comparable among the other B subclasses. These results were also reported in Tong et al study [9].

Concerning aberrant T/B lymphoid antigens, we reported 4 (2.5%) cases of B-ALL with T markers and 2 (2%) cases of T-ALL with B markers. Sharm et al noted higher rates evaluated at 14.1 % for aberrant T antigens (CD4 and CD7 in 11.6% and 2.5% of cases, respectively) and 19.5% of aberrant B antigens (CD19 and CD79a in 7% and 12.5%, respectively) [13]. In fact, frequency of this aberrant phenotype in ALL is not well defined because authors generally focused in their trials on the assessment of MyAg. Importantly, It has been noted that aberrant expression of T-antigen in B-ALL harbor a poor prognosis as it predicts patients at increased risk of relapse [37].

In this present study, we spread out immunophenotyping of adult patients with ALL since the introduction of flow cytometry analysis in our center until Juanuary 2020. Currently, we installed an 8- color flow cytometry Becton Dickinson (BD) FACSLyric ^{TM} and we aim to follow standardized flow cytometry procedures and antibody panels as recommended by the Euroflow consortium [38]. This standardized approach would increase reproducibility and improve comparability of immunophenotypic data between clinical studies in the current years.

CONCLUSION

Immunophenotopic profiles of our adult ALL share many findings reported in literature with some particularities: cortical T-ALL and pre-B ALL are the most common subtypes, aberrant phenotypes expression seems to be less common compared to other series from different ethnic populations and CD33 is the most frequent aberrant myeloid antigen followed by CD13. A wider trial optimally multicenter in which a standardized flow cytometry assessment approach is adopted seems warranted in order to better assess the phenotypic profiles of our population, improve the comparability with other studies and evaluate the impact of the immunophenotypic particularities on clinical presentation and prognosis.

Conflict of interest:
The authors declare no conflict of interest.

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