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Impact of Aberrant Antigens in the Outcome of Patients with Acute Leukemia at a Referral Institution in Mexico City

Sergio Rodríguez-Rodríguez[1,2], Alan Pomerantz[1,2], Roberta Demichelis-Gómez[1], Georgina Barrera-Lumbreras[1], Olga V. Barrales-Benítez[1], Xavier Lopez-Karpovitch[1] and Álvaro Aguayo[1]*

[1]Department of Hematology and Oncology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City; [2]Faculty of Health Sciences, Universidad Anáhuac México Norte, Huixquilucan. Mexico

ABSTRACT

Background: Patients with acute leukemia can express aberrant markers, defined as antigens that are normally restricted to a different lineage. The reported significance and frequency of these markers is inconclusive. We assessed the frequency and impact of aberrant markers in patients with acute leukemia in a referral institution in Mexico City. **Methods:** We included 433 patients, diagnosed and treated between 2005 and 2015 in our institution. **Results:** Aberrant markers were expressed in 128 patients (29.6%); CD13 and CD33 were the most frequent aberrant markers in patients with acute lymphoblastic leukemia, while CD7 and CD19 were the most frequent in patients with acute myeloid leukemia. In the univariate analysis, the group with aberrant markers had a lower disease-free survival when compared with the aberrant-free group (8 vs. 13 months) ($p = 0.03$). Aberrant expression of CD10, CD20, and CD33 correlated with a worse outcome in a statistically significant manner. In the multivariate analysis, male gender, lymphoid lineage, secondary leukemia, high risk at diagnosis, and the presence of aberrant markers had a significantly negative impact on disease-free survival. **Conclusion:** The use of more aggressive treatment strategies could be considered in patients with acute leukemia and an aberrant expression of CD10, CD20, and CD33. (REV INVES CLIN. 2016;68:305-13)

Key words: Aberrant marker. Acute leukemia. Acute lymphoblastic leukemia. Acute myeloid leukemia. Flow cytometry.

INTRODUCTION

The adequate classification of acute leukemias (AL) is of paramount importance to provide the patient with the best possible treatment. In the past, ALs were classified based on morphological and cytochemical features. More recently, new classification schemes have arisen based on the implementation of immunophenotypic, cytogenetic, and molecular markers[1]. Flow cytometry performs a fast and comprehensive determination of antigen expression in ALs and, alongside the morphological features of the blast cells, it can

Corresponding author:
*Álvaro Aguayo
Departamento de Hematología y Oncología
Instituto Nacional de Ciencias Médicas y Nutrición
Salvador Zubirán
Vasco de Quiroga, 15
Col. Belisario Domínguez, Sección XVI, Del. Tlalpan
C.P. 14080, Ciudad de México, México
E-mail: alvaroaguayo@hotmail.com

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provide a definitive diagnosis and discriminate between the different types of ALs, which are: acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), and acute leukemia of ambiguous lineage that includes mixed phenotype acute leukemia (MPAL) and acute undifferentiated leukemia (AUL)[1,2].

In addition to detecting the antigens that allow for the correct classification of ALs, flow cytometry has also aided in the identification of aberrant markers. These are antigens that are normally expressed in a certain lineage, but in some instances are expressed on the blasts of an AL that belongs to another lineage (i.e. CD13 in ALL)[3]. In the medical literature, the reported frequency of this phenomenon is highly variable[3,4]. For example, in one study it was reported that CD13, a myeloid-related antigen, was present in 22% (n = 6/27) of ALL cases, while in other studies the expression of CD13 as an aberrant marker was reported in 53% (n = 10/71) and 37% (n = 46/124) of patients with ALL[5-7]. Moreover, no consensus has been reached about the clinical significance of this phenomenon. Some authors have demonstrated a lower disease-free survival (DFS) and overall survival (OS) in ALL patients when CD13 is present as an aberrant marker[8,9]. Other studies have reported no prognostic impact or a favorable outcome when CD13 is expressed in ALL patients[10,11].

With the aim of expanding the knowledge about this phenomenon, we retrospectively assessed the frequency and prognostic impact of aberrant markers in 433 Mexican patients with AL diagnosed and treated at a referral academic institution in Mexico.

MATERIALS AND METHODS

Patients

All the patients with AL diagnosed and treated between January 2005 and June 2015 at the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ) were included in this study. The diagnosis of AL and its classification were based initially on the French-American-British (FAB) scheme, and later the classification proposed by the World Health Organization (WHO) in 2008[12,13]. A 20% blast threshold was used for the diagnosis of AL; blast percentages were derived from peripheral blood smears or from blood marrow samples stained with Wright-Giemsa[13].

The following data were gathered from the patients' medical record: date of diagnosis, laboratory results corresponding with the date of diagnosis, the immunophenotypic features of bone marrow or peripheral blood samples, and the performance status of each patient according to the Eastern Cooperative Oncology Group (ECOG) Scale.

By using international criteria (i.e. leukocytes and age at diagnosis, cytogenetics), patients were stratified according to their risk and were divided into non high-risk and high-risk groups[14]. Patients with incomplete data and those who were diagnosed or followed up at another institution were excluded from the study.

The following parameters were recorded: occurrence of complete remission (CR) and relapse, as well as DFS and OS. Complete remission was defined as the presence of < 5% of blasts in the bone marrow aspirate one month after the induction therapy was initiated (+28 days), along with the absence of blasts in peripheral blood, no extramedullary leukemia infiltrations, an absolute neutrophil count ≥ 1×10^9/l, and platelet counts ≥ 100×10^9/l[15,16]. Disease-free survival was defined as the interval between CR and relapse, and OS was defined as the interval between diagnosis and last follow-up date or date of death from any cause.

Lastly, the patients were stratified into two groups: patients without aberrant markers and patients with aberrant markers. We analyzed if there was any difference in the clinical, laboratory, and survival parameters between these two groups.

Treatment

Three main strategies were used in the treatment of patients diagnosed with ALL during this 10-year period: (i) hyper-fractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone (hyper-CVAD) and derived regimens (i.e. hyper-CVAD plus rituximab or imatinib, when appropriate); (ii) local or institutional regimens modified from the German Multicenter Study Group for ALL (GMALL) protocol[17]; and (iii) pediatric-based therapies for adults (i.e. the Dana-Farber Cancer Institute ALL Consortium Protocol 95-01).

Regarding the treatment of patients with AML, we used the typical 7+3 protocols (cytarabine and

doxorubicin/daunorubicin), and the all-trans retinoic acid (ATRA)-based regimens for acute promyelocytic leukemias. Since November 2010, we started to introduce more intensive induction therapies in patients with AML, including the 7+3+7 protocol (adding etoposide) and intermediate or high doses of cytarabine (IDAC and HIDAC, respectively).

Ethics approval

The study was approved by the institutional review boards of ethics and research of our institution.

Flow cytometry

Ethylenediaminetetraacetic acid anticoagulated blood, bone marrow, or both specimens were processed and stained using a pre-lysing technique with a variable combination of the following antibodies: CD2, surface CD3 (sCD3), cytoplasmic CD3 (cCD3), CD5, CD7, CD10, CD11b, CD13, CD14, CD15, CD19, CD20, CD22, CD33, CD34, CD41, CD56, CD61, CD64, cCD79a, CD117, CD235a, anti-MPO, HLA-DR, sIgM, cIgM. An eight-color flow cytometry panel was used, which included the following fluorochromes: phycoerythrin (PE), fluorescein isothiocyanate (FITC), peridin chlorophyll (PerCP), allophycocyanin (APC), V450 and V500, PE and cyanine dye 7 (PE-Cy7), and PerCP-Cy5.5. Data were obtained and analyzed on a FACSCanto™ II flow cytometer (Becton Dickinson Immunocytometry Systems, San José, CA, USA) with the aid of the FACSDIVA™ software (Becton Dickinson, San José, CA, USA). The samples were analyzed using flow cytometry with a CD45 gating technique, which allowed the analysis of only the blast population. The expression of cytoplasmic markers (MPO, CD3, CD79a and IgM) and CD34 was considered positive if these markers were present in 10% or more of the blast population, 20% or more of the blast population for myeloid markers and HLA-DR, and 30% or more for lymphoid markers[18].

Statistical analysis

For categorical parameters, the two-tailed Chi-square and the Fisher exact tests were used for group comparison. For continuous variables, the Mann-Whitney, Kruskal-Wallis, and the median test were used to compare non-normal data between groups. For the survival analysis, the log-rank test was used in the univariate analysis, and the Cox regression test was used for the multivariate analysis. Statistical analysis was performed with the SPSS Software package, v21.0 (SPSS Inc., Chicago, USA).

RESULTS

Clinical and laboratory features

A total of 433 patients with AL were diagnosed and treated at our institution between 2005 and 2015. Acute lymphoblastic leukemia was diagnosed in 216 patients (49.9%), AML in 208 (48%), and MPAL in nine (2.1%). The median follow-up was seven months (range 0-123). Patients were divided into two groups: a group characterized by the presence of aberrant markers and another group without such markers. The clinical and laboratory characteristics at the time of diagnosis are shown in table 1; there were no statistical significant differences between both groups at diagnosis.

Association of aberrant markers with clinical outcomes

Expression profile of aberrant markers

One hundred and twenty-eight (128/433, 29.6%) patients with AL expressed aberrant markers, distributed as follows: 86 patients (19.9%) expressed one marker, 24 (5.5%) expressed two, and nine (2.1%) expressed three or more markers. The other nine patients (2.1%) were diagnosed with MPAL, and their blast cells expressed both myeloid and B-cell markers.

Expression of aberrant markers in acute lymphoblastic leukemia

In the 64 patients (64/216, 29.7%) who were diagnosed with ALL and expressed aberrant markers, these antigens were distributed as follows: 53 (82.8%) expressed myeloid markers, eight B-cell ALL (12.5%) expressed myeloid and T-cell markers simultaneously, two B-cell ALL (3.1%) expressed T-cell markers, and one T-cell ALL (1.6%) expressed a B-cell marker. Of the 57 patients that had B-cell ALL, the most frequent aberrant markers were CD13, CD33, CD15 and CD2. In the seven patients with T-cell ALL, CD13 and CD33 were also the most frequent markers. Detailed data of aberrant markers expression is presented in table 2.

Table 1. Demographic and clinical characteristics of patients

	Without aberrant markers No. patients (%) (n = 305)	With aberrant markers No. patients (%) (n = 128)	p
Gender			0.916
Male	167 (54.8)	71 (55.5)	
Female	138 (45.2)	57 (44.5)	
Age, years			
Median (range)	40 (15-88)	39 (16-85)	0.96
≥ 65	48 (15.7)	18 (14.1)	0.77
Cell lineage			0.517*
ALL	152 (49.8)	64 (50)	
AML	153 (50.2)	55 (43)	
MPAL	-	9 (7)	
Leukemia etiology			0.445
De novo	282 (92.5)	115 (89.8)	
Secondary	23 (7.5)	13 (10.2)	
Risk			0.516
Non-high risk	192 (63)	76 (59.4)	
High risk	113 (37)	52 (40.6)	
ECOG			0.458
0	43 (14.1)	14 (10.9)	
1-2	193 (63.3)	73 (57)	
3-4	8 (2.6)	3 (2.3)	
Hemoglobin, g/dl			0.964
Median (range)	8.19 (3.4-15.4)	8.3 (4.8-14.6)	
Leukocyte count, $\times 10^9$/l			0.52
Median (range)	7.5 (0.1-422.9)	5.75 (0.2-337.0)	
Platelet count, $\times 10^9$/l			0.665
Median (range)	38 (5-1,123)	33 (0-472)	
Blasts, %			
PB, median (range)	44.5 (1-98)	62 (5-100)	0.763
BM, median (range)	48 (1-98)	61.6 (2-94)	0.788

* Fisher's exact test was used for the comparison of the presence of antigen markers between acute myeloid leukemia and acute lymphoblastic leukemia only.
ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; BM: bone marrow; ECOG: Eastern Cooperative Oncology Group; MPAL: mixed-phenotype acute leukemia; PB: peripheral blood.

Expression of aberrant markers in acute myeloid leukemia

Of the 55 patients (55/208, 26.4%) who were diagnosed with AML and expressed lymphoid markers, 31 (56.4%) expressed T-cell markers, 19 (34.5%) expressed B-cell markers, and five (9.1%) expressed both T-cell and B-cell markers. The most frequent lymphoid markers in AML were CD7, CD19, CD2, and CD22.

Association of aberrant markers with complete remission and relapse rates

Of the 433 patients with AL, 242 (55.9%) achieved CR, 140 (32.3%) relapsed at least once, and 293 patients (67.7%) died. The differences between the groups with and without aberrant markers are shown in table 3. The only statistically significant difference between the two groups was the number of deaths: 64.6% in the aberrant-free group vs. 75% in the individuals with aberrant markers (p = 0.042).

Association of aberrant markers with overall and disease-free survival

In the univariate analysis, the median OS of the group without aberrant markers was 12 months (range 0-114) vs. eight months (0.07-123) in the group with aberrant markers (p = 0.06); median DFS in the group without markers was 13 months (range 0-109) vs. eight months (0-84) in the group with aberrant markers (p = 0.03) (Fig. 1). In the univariate analysis, the variables that had a negative impact on OS were: age

Table 2. Expression of aberrant markers in acute leukemia

Aberrant Marker	AML No. patients (%) (n = 55)	B-cell ALL No. patients (%) (n = 57)	T-cell ALL No. patients (%) (n = 7)
CD2	9 (16.4)	6 (10.5)	–
CD5	1 (1.8)	1 (1.7)	–
CD7	21 (38.2)	5 (8.8)	–
CD10	3 (5.5)	–	–
CD11b	–	5 (8.8)	2 (28.6)
CD13	–	37 (64.9)	6 (85.7)
CD14	–	1 (1.7)	0
CD15	–	15 (26.3)	2 (28.6)
CD19	11 (20.0)	–	0
CD20	1 (1.8)	–	0
CD22	8 (14.5)	–	0
CD33	–	18 (31.6)	3 (42.8)
CD41a	–	1 (1.7)	0
CD56	6 (10.9)	1 (1.7)	–
CD64	–	1 (1.7)	0
CD79a	6 (10.9)	–	1 (14.3)
CD117	–	5 (8.8)	2 (28.6)

ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia.

at diagnosis (≥ 65 years), etiology of AL (secondary leukemia), risk at diagnosis (high risk), initial ECOG (≥ 3), and lack of CR (Table 4).

For DFS, the variables that negatively affected this clinical endpoint were: gender (male), cell lineage (lymphoid), etiology of AL (secondary), risk at diagnosis (high risk), and, as stated before, the presence of aberrant markers. In the multivariate analysis, which included all of the variables that had a statistically significant impact, only DFS was affected in a statistically significant manner by the presence of aberrant markers (Table 4).

Table 3. Complete remission, relapse, and overall and disease-free survival rates

	Without aberrant markers No. patients (%) (n = 305)	With aberrant markers No. patients (%) (n = 128)	p
Complete remission	170 (55.7)	72 (56.3)	1.000
Relapse	95 (31.1)	45 (35.2)	0.432
Deceased	197 (64.6)	96 (75.0)	0.042

When comparing the number of aberrant markers on the leukemic blast population, no statistically significant association was observed between the patients that had one, two, or three or more aberrant antigens and DFS or OS. However, in patients with three or more aberrant markers, there was a trend towards a shorter DFS and OS (p = 0.06 and p = 0.059, respectively).

Association of specific aberrant markers with overall and disease-free survival

The individual analysis of specific aberrant markers showed a lower OS in patients with AML that expressed CD20 (nine months in CD20-negative vs.

Figure 1. Association of aberrant markers with overall survival **(A)** and disease-free survival **(B)** (log-rank test).

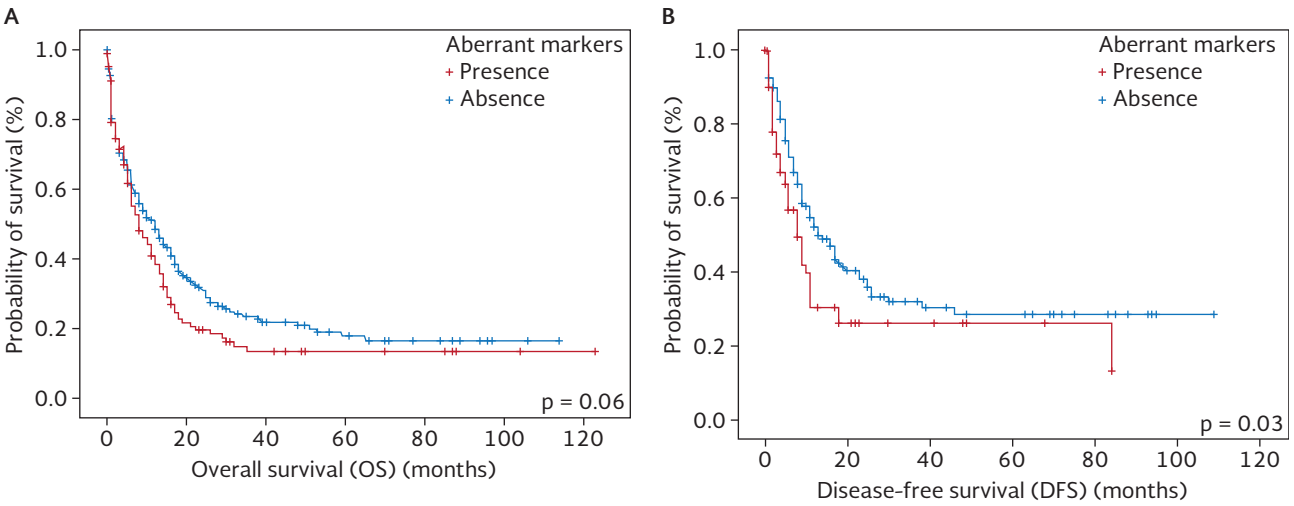

Table 4. Univariate and multivariate analysis of factors influencing overall survival

Variables	OS, months	Univariate	Multivariate	
	Median (range)	p	HR (95% CI)	p
Age, years		< 0.001	0.79 (0.548-1.444)	0.213
< 65	13 (0-123)			
≥ 65	3 (0-28)			
Gender		0.170		
Lineage		0.163		
Etiology		< 0.001	1.058 (0.673-1.665)	0.806
De novo	12 (0-123)			
Secondary	5 (0.23-30)			
Risk		0.042	1.098 (0.836-1.441)	0.502
Non-high risk	12 (0-114)			
High risk	8 (0-123)			
ECOG		< 0.001	1.447 (1.204-1.704)	< 0.001
0	17 (0-84)			
1-2	10 (0-54)			
3-4	8 (0-12)			
Aberrant markers		0.06		
Complete remission		< 0.001	0.134 (0.096-0.188)	< 0.001
Not achieved	2 (0-48)			
Achieved	19 (1-123)			

ECOG: Eastern Cooperative Oncology Group; OS: overall survival.

0.13 months in CD20-positive) (p < 0.001) and in patients with ALL that expressed CD33 (13 months in CD33-negative vs. six months in CD33-positive) (p = 0.01). Additionally, DFS was shorter in the patients with AML that expressed CD10 (18 months in CD10-negative vs. two months in CD10-positive) (p = 0.001) (Fig. 2).

Other associations

We also analyzed the adjusted prognostic value of the aberrant antigens in the non high-risk and high-risk groups. For OS, we found no significant differences in the median survival between the non high-risk group and the high-risk group (p = 0.066) (Table 4). Regarding DFS, we found a statistically significant difference between the non high-risk group and the high-risk group (p = 0.047) (Table 5).

Impact of the treatment regimens

Of the 216 patients diagnosed with ALL, 196 (90.7%) received a proper induction therapy, divided as follows: 112 patients (57.1%) received the hyper-CVAD or derived regimens, 56 (28.6%) received local or institutional regimens, 11 (5.6%) were treated with pediatric based protocols, and 17 patients (8.7%) received other induction protocols. When adjusting the prognostic value of the aberrant antigens between the different treatments used, we found no significant differences in OS and DFS in the patients treated with hyper-CVAD or derived regimens. On the other hand, DFS was significantly different between patients that received the local or institutional regimens (p = 0.044) (Table 6).

Regarding the patients with AML, 137 (65.9%) received an induction therapy as follows: 81 (59.1%) received a typical 7+3 protocol, 17 (12.4%) received an intensive induction protocol (either 7+3+7 or IDAC/HIDAC), and 18 patients (13.1%) received other induction protocols. The remaining 21 patients (15.4%) had acute promyelocytic leukemia and all received an ATRA-based regimen. When adjusting the prognostic value of the aberrant antigens between the different treatments used, we found no significant differences in OS or DFS.

DISCUSSION

In our study, aberrant antigens were expressed in 27.5% of patients with AL (27.8% of ALL and 26.4% of AML patients; p = 0.517). In both, B-cell and T-cell

Figure 2. Aberrant expression of CD20 in acute myeloid leukemia **(A)** and CD33 in acute lymphoblastic leukemia **(B)** and their association with overall survival; and CD10 in acute myeloid leukemia, **(C)** and its association with disease-free survival (log-rank test).

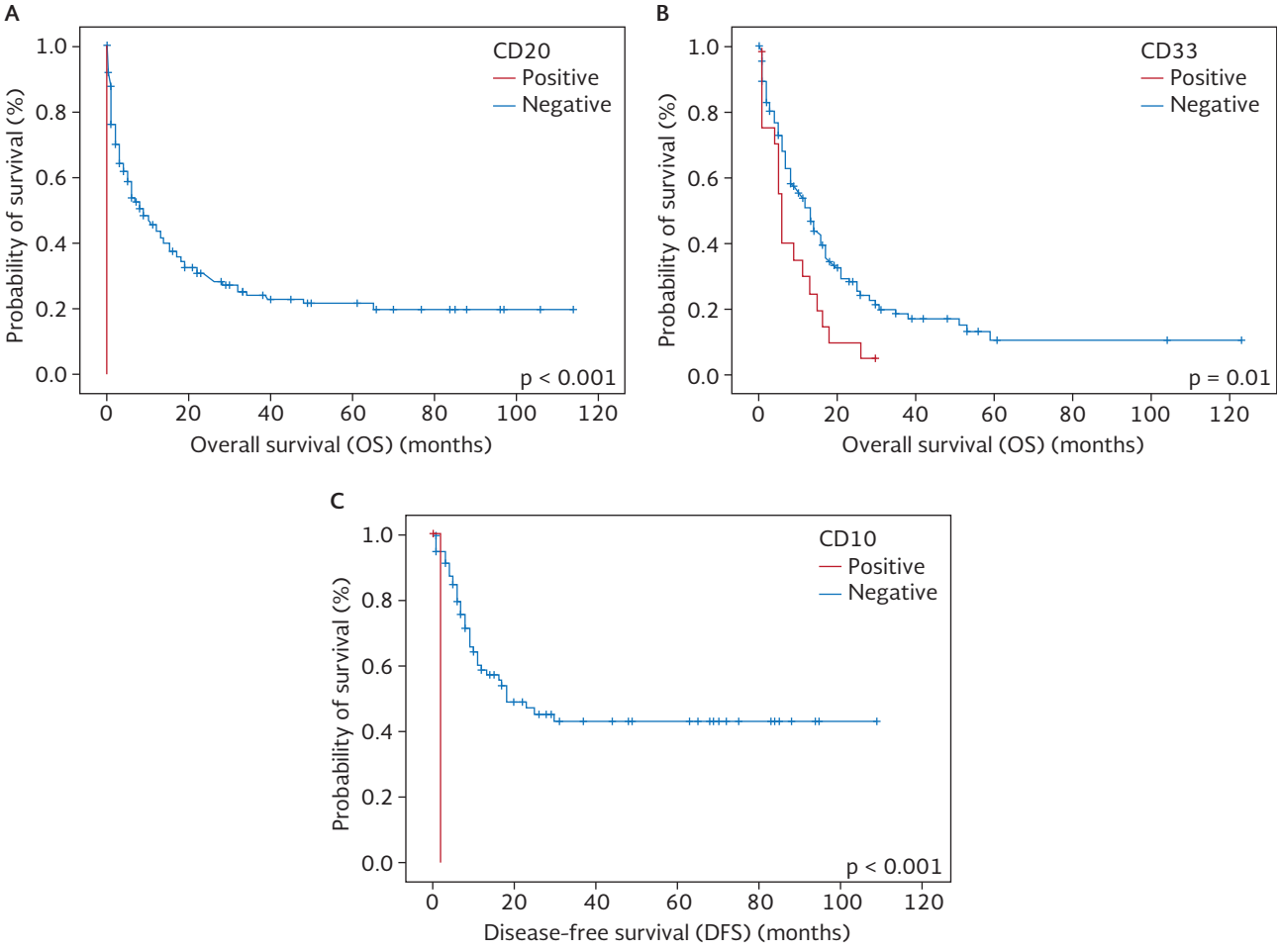

Table 5. Univariate and multivariate analysis of factors influencing disease-free survival

Variables	DFS, months	Univariate	Multivariate	
	Median (range)	p	HR (95% CI)	p
Age		0.647		
Gender		0.025	0.678 (0.483-0.952)	0.025
Male	9 (0-94)			
Female	17 (0-109)			
Lineage		< 0.001	0.911 (0.637-1.305)	0.612
Lymphoid	9 (0-93)			
Myeloid	18 (0-109)			
Etiology		0.065		
Risk		< 0.001	2.452 (1.694-3.551)	< 0.001
Non-high risk	17 (0-109)			
High risk	6 (0-84)			
ECOG		0.179		
Aberrant markers		0.03	1.488 (1.040-2.127)	0.03
Absence	13 (0-109)			
Presence	8 (0-84)			

DFS: disease-free survival; ECOG: Eastern Cooperative Oncology Group.

Table 6. Prognostic value of aberrant markers adjusted to risk at diagnosis and treatment protocols

Variables	OS, months Median (range)	p	DFS, months Median (range)	p
Risk at diagnosis		0.066		0.047
Non-high risk without aberrant markers	13 (0-114)		23 (0-109)	
Non-high risk with aberrant markers	11 (0.67-88.0)		11 (0-84)	
High risk without aberrant markers	11 (0-65)		8 (0-63)	
High risk with aberrant markers	7 (0.13-123.0)		3 (0-84)	
ALL protocols				
Hyper-CVAD regimens without aberrant markers	14 (1-71)	0.656	12 (0-70)	0.578
Hyper-CVAD regimens with aberrant markers	15 (67-104)		11 (0-84)	
Institutional regimens without aberrant markers	16 (1-94)	0.234	11 (0-91)	0.044
Institutional regimens with aberrant markers	6 (0.4-123.0)		3 (0-41)	
AML protocols				
Intensive regimens without aberrant markers	25 (1-38)	0.501	30 (0-37)	0.538
Intensive regimens with aberrant markers	19 (14-50)		11 (3-49)	
7+3 without aberrant markers	13 (0.2-106.0)	0.655	13 (0-95)	0.446
7+3 with aberrant markers	6 (0.46-88.0)		11 (0-84)	

ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; DFS: disease-free survival; Hyper-CVAD: hyper-fractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone; OS: overall survival; 7+3: cytarabine and doxorubicin/daunorubicin.

ALL, CD13 and CD33 were the most common aberrant markers, and in patients with AML the most common were CD7, CD19, CD2 and CD22. As stated before, no consensus exists on the frequency of aberrant markers, as exemplified by the lack of agreement in several studies[19-26].

A statistically significant association has been reported between the presence of myeloid markers in adult ALL and a lower CR rate, as well as a shorter survival[27]. In our study, in addition to a higher number of deaths, patients with aberrant markers had an inferior DFS when compared with the aberrant-free individuals in both the univariate and multivariate analyses. It is important to state that in the multivariate analysis, the inferior DFS was statistically significant in spite of the presence of certain factors that, in the univariate analysis, predicted a worse outcome. Furthermore, a trend was observed in our results regarding a lower DFS when three or more aberrant markers were present.

The presence of CD33 as an aberrant marker in patients with ALL has been related with a poor prognosis or with a lack of any clinical impact[28]. Other researchers have shown an association between the presence of CD7, CD56, and CD79 as aberrant markers and a poor outcome in patients with AML[29,30]. In the present study, AML patients expressing CD20 and ALL patients expressing CD33 as aberrant markers achieved a shorter OS than the survival attained by the aberrant-free group. Additionally, AML patients had a shorter DFS when CD10 was expressed as an aberrant marker.

One of the advantages of this study is the inclusion of a rather large adult population with AL expressing aberrant markers. Furthermore, when comparing the prognostic value of the aberrant markers adjusted by the risk at diagnosis and by the use of the different induction chemotherapies, we found that patients who presented aberrant markers and were categorized as high risk at diagnosis, or were treated with local or institutional regimens, had a worse median DFS than those with the same characteristics but without aberrant markers. Therefore, we can state that the use of hyper-CVAD should be preferred over the local or institutional regimens when treating patients with ALL who present aberrant markers. Moreover, based on this research, the use of aggressive treatment strategies (i.e. as in high-risk individuals) could be suggested in the aforementioned patients, as well as AML patients expressing CD10 and CD20 and ALL patients expressing CD33[31,32].

Based on the fact that the literature is inconclusive about the significance and frequency of this phenomenon, and considering that this study had some limitations due to its retrospective nature and to the use of rather heterogeneous induction regimens, we cannot

suggest the immediate translation of our results into everyday clinical practice. Thus, to reach a consensus on the meaning and frequency of this phenomenon, we suggest that future research could follow these recommendations: (i) uniformity in the threshold for positivity of the various antigens; (ii) standardization of the monoclonal antibodies used to detect these antigens; (iii) use of similar populations in a given study; and (iv) use of similar chemotherapeutic regimens, which might affect the outcome of patients with AL when interpreting the prognostic significance of an aberrant marker[33].

REFERENCES

1. Peters JM, Ansari MQ. Multiparameter flow cytometry in the diagnosis and management of acute leukemia. Arch Pathol Lab Med. 2011;135:44-54.
2. Manivannan P, Puri V, Somasundaram V, et al. Can threshold for MPO by flow cytometry be reduced in classifying acute leukaemia? A comparison of flow cytometric and cytochemical myeloperoxidase using different flow cytometric cut-offs. Hematology. 2015;20:455-61.
3. Griesinger F, Pir´-Noack M, Kaib N, et al. Leukaemia-associated immunophenotypes (LAIP) are observed in 90% of adult and childhood acute lymphoblastic leukaemia: Detection in remission marrow predicts outcome. Br J Haematol. 1999;105:241-55.
4. Sharma RK, Purohit A, Somasundaram V, et al. Aberrant myeloid antigen co-expression is correlated with high percentages of CD34-positive cells among blasts of acute lymphoblastic leukemia patients: An Indian tertiary care center perspective. Blood Res. 2014;49:241-5.
5. Suggs JL, Cruse JM, Lewis RE. Aberrant myeloid marker expression in precursor B-cell and T-cell leukemias. Exp Mol Pathol. 2007;83:471-3.
6. Mazher N, Malik N, Imran A, Chughtai O, Chughtai AS. Aberrant expression of CD markers in acute leukemia. Ann Pakistan Inst Med Sci. 2013;9:99-102.
7. Lahjouji A, Bachir F, Bennani S, Quessar A, Amzazi S. The immunophenotype of adult T acute lymphoblastic leukemia in Morocco. Exp Oncol. 2015;37:64-9.
8. Dalal BI, Al Mugairi A, Pi S, et al. Aberrant expression of CD13 identifies a subgroup of standard-risk adult acute lymphoblastic leukemia with inferior survival. Clin Lymphoma Myeloma Leuk. 2014;14:239-44.
9. Craddock KJ, Chen Y, Brandwein JM, Chang H. CD13 expression is an independent adverse prognostic factor in adults with Philadelphia chromosome negative B cell acute lymphoblastic leukemia. Leuk Res. 2013;37:759-64.
10. Yenerel MN, Atamer T, Yavuz AS, et al. Myeloid antigen expression provides favorable outcome in patients with adult acute lymphoblastic leukemia: a single-center study. Ann Hematol. 2002;81:498-503.
11. Vitale A, Guarini A, Ariola C, et al. Results of the GIMEMA ALL 0496 trial. Haematologica. 2007;92:342-8.
12. Bennett JM, Catovsky D, Daniel M-T, et al. Proposals for the classification of the acute leukemias. Br J Haematol. 1976;33: 451-8.
13. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the WHO classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009;114:937-52.
14. Ganzel C, Rowe JM. Prognostic factors in adult acute leukemia. Hematol Oncol Clin North Am. 2011;25:1163-87.
15. Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the International Working Group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. J Clin Oncol. 2003;21:4642-9.
16. Demichelis-Gómez R, Crespo-Solís E, Pérez-Jacobo LF, Valencia-Rocha UR, Rosas-López A. Outcomes of substituting oral fludarabine for Intravenous fludarabine in combination with cytarabine and filgrastim for treatment of primary refractory or relapsed acute leukemias. Rev Invest Clin. 2015;67:287-95.
17. Arteaga-Ortiz L, Buitrón-Santiago N, Rosas-López A, et al. [Acute lymphoblastic leukemia: experience in adult patients treated with hyperCVAD and 0195 Protocol, at the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. Cohort 2003-2007]. Rev Invest Clin. 2008;60:459-69.
18. Chiaretti S, Zini G, Bassan R. Diagnosis and subclassification of acute lymphoblastic leukemia. Mediterr J Hematol Infect Dis. 2014;6:1-14.
19. Sobol R, Mick R, Roystno I, et al. Clinical importance of myeloid antigen expression in adult acute lymphoblastic leukemia. N Engl J Med. 1987;316:1111-7.
20. Bahia DM, Yamamoto M, Chauffaille MD, et al. Aberrant phenotypes in acute myeloid leukemia: a high frequency and its clinical significance. Haematologica. 2001;86:801-6.
21. Lopes TC, Andrade KN, Camelo NL, Rodrigues VP, Oliveira RA. Influence of aberrant myeloid expression on acute lymphoblastic leukemia in children and adolescents from Maranhao, Brazil. Genet Mol Res. 2014;13:10301-7.
22. Cezar RS, Cerqueira BA, da Paz Sde S, et al. Outcome of B-cell acute lymphoblastic leukemia in Brazilian children: Immunophenotypical, hematological, and clinical evaluation. J Pediatr Hematol Oncol. 2015;37:423-8.
23. Smith FO, Lampkin BC, Versteeg C, et al. Expression of lymphoid-associated cell surface antigens by childhood acute myeloid leukemia cells lacks prognostic significance. Blood. 1992;79:2415-22.
24. Abdelhaleem M. Frequent but nonrandom expression of myeloid markers on de novo childhood acute lymphoblastic leukemia. Exp Mol Pathol. 2007;83:138-41.
25. Seegmiller AC, Kroft SH, Karandikar NJ, McKenna RW. Characterization of immunophenotypic aberrancies in 200 cases of B acute lymphoblastic leukemia. Am J Clin Pathol. 2009;132:940-9.
26. Lewis RE, Cruse JM, Sanders CM, Webb RN, Suggs JL. Aberrant expression of T-cell markers in acute myeloid leukemia. Exp Mol Pathol. 2007;83:462-3.
27. Urbano-Ispizua A, Matutes E, Villamor N, et al. Clinical significance of the presence of myeloid associated antigens in acute lymphoblastic leukaemia. Br J Haematol. 1990;75:202-7.
28. Mejstríková E, Kalina T, Trka J, et al. Correlation of CD33 with poorer prognosis in childhood ALL implicates a potential of anti-CD33 frontline therapy. Leukemia. 2005;19:1092-4.
29. Ossenkoppele GJ, van de Loosdrecht AA, Schuurhuis GJ. Review of the relevance of aberrant antigen expression by flow cytometry in myeloid neoplasms. Br J Haematol. 2011;153:421-36.
30. Breccia M, De Propris MS, Minotti C, et al. Aberrant phenotypic expression of CD15 and CD56 identifies poor prognostic acute promyelocytic leukemia patients. Leuk Res. 2014;38:194-7.
31. Schiller GJ. High-risk acute myelogenous leukemia: treatment today ... and tomorrow. Hematology. 2013;2013:201-8.
32. Bhojwani D, Howard SC, Pui C-H. High-risk childhood acute lymphoblastic leukemia. Clin Lymphoma Myeloma. 2009;9:S222-30.
33. Firat H, Favier R, Adam M, et al. Determination of myeloid antigen expression on childhood acute lymphoblastic leukaemia cells: discrepancies using different monoclonal antibody clones. Leuk Lymphoma. 2001;42:75-82.