Original Article

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Aberrant Immunophenotype in Childhood Acute Lymphoblastic Leukemia: Overall Survival and Disease-Free Survival up to 18 Months after Diagnosis

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Abstract

Background: Aberrant immunophenotype, characterized by the expression of lineage-inappropriate antigens on leukemic blasts, is associated with early relapse and a poor survival rate in Acute Lymphoblastic Leukemia (ALL). Early relapse gives a bad prognosis for ALL. This study evaluated the association between aberrant immunophenotype and Overall Survival (OS) and Disease-Free Survival (DFS) up to 18 months following the diagnosis of ALL.

Materials and Methods: A retrospective cohort study was conducted to identify aberrant immunophenotype in 156 patients under 18 years of age with ALL. In this study, done at a tertiary level hospital, OS and DFS were analyzed using the Kaplan-Meier method, the Breslow test and a Cox regression model, with hazard ratios to determine the variables associated with mortality and relapse over an 18-month follow-up period.

Results: The mean age at diagnosis was 6.5 years, and 57% of the patients were male. Aberrant immunophenotype was expressed in 87 patients. After 18 months of follow-up, 41 patients had died. The CD123 antigen was mostly associated with relapses (p=0.010). CD66c and CD123 co-expression decreased OS (p=0.040) and DFS (p=0.003). The univariate analysis demonstrated that CD66c and CD123 co-expression (p=0.005), single CD123 expression (p=0.001), and positive Measurable Residual Disease (MRD) (p=0.001) increased the risk of relapse. In a multivariable analysis, only risk stratification and MRD remained as independent predictors of OS and DFS.

Conclusion: CD66c and CD123 co-expression was associated with decreased OS and DFS and a higher risk of relapse. However, this association did not remain significant in the multivariate model, indicating that it is not an independent prognostic factor. Nonetheless, isolated CD123 expression was linked to positive MRD and an increased risk of relapse. These observations provide clinically relevant preliminary insights, particularly in settings with survival disparities. These require confirmation in larger cohorts with extended follow-up and integrated molecular data.

Keywords: Childhood ALL, Disease-Free Survival, Immunophenotyping, Leukemia, Prognosis

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Introduction

The aberrant leukemic cells can express surface and cytoplasmic antigens that differ from those of normal hematopoiesis and may even display antigens from another cell lineage (1, 2). These characteristics of leukemic cell precursors are a consequence of many genetic alterations, which are associated with the well-identified mutation profiles of both good and poor prognosis (3, 4).

The existence of the aberrant immunophenotype in childhood acute lymphoblastic leukemia (ALL) has an incidence of about 50% in both Latin American and North American populations (5). Although the standard follow-up time for overall survival (OS) and disease-free survival (DFS) is 3-5 years, the first 18 months are crucial since, in this period of time, the physician can identify clinical and biological characteristics of the disease related to very early relapse (6, 7). The objective of the present study was to analyze the association of the aberrant immunophenotype with OS and DFS up to 18 months after diagnosis in the Bajio region of Mexico.

Material and Methods

Study design

This retrospective cohort study was conducted at the Sub-specialty Medical Unit, Hospital of Gynecology and Pediatrics # 48, Mexican Institute of Social Security, in a pediatric hematology service from January 2018 to June 2024. The patients were of both sexes under 18 years of age. They all had a diagnosis of ALL and were under treatment in the pediatric hematology service. The patients with incomplete records and those who did not carry on with their medical consultations were excluded from the study.

Data collection

Sociodemographic characteristics such as place of origin, sex and age were recorded. The patients were classified in the high-risk ALL group if they had at least one of the following characteristics: age < 1 year or ≥ 10 years, leukocyte count ≥ 50x109/L, central nervous system (CNS) disease 2 (less than 5 blasts/mL) or CNS disease 3 (greater than 5 blasts/mL) determined with lumbar puncture, prednisone poor response (PPR), which is for patients with a blast count in peripheral blood > 11x109/L on day 8 of treatment, T-cell ALL lineage, and positive measurable residual disease (MRD) at the end of induction therapy. The patients who did not meet any of these criteria were assigned to a standard-risk ALL group (8).

The immunophenotype of every patient was analyzed to determine the expression of aberrant antigens. The immunophenotype reports were collected from physical and electronic clinical records. The antigens were expressed as positive, weak positive or negative, according to the quality standards of the Childhood Cancer Cytomics laboratory, ONCOCREAN Reference Laboratory of the Eastern Biomedical Research Center of the Mexican Social Security Institute in Puebla, Mexico. For immunophenotype testing, bone marrow samples were collected and stained for flow cytometry analysis following the EuroFlow™ guidelines and protocols. First, the samples were stained using the acute leukemia targeting tube (ALOT) to determine the lineage of immature blast cell populations. Subsequently, staining was performed with extended panels for acute leukemia classification; up to 60 different markers were used for the identification of blasts. According to the affected cell lineage, the protocols have the ability to classify ALL into six categories including ProB-ALL (CD34+ CD19+ cyCD79a+), ProB-PreB-ALL (CD34-/+ CD19+ cyCD79a+), PreB-ALL (CD34- CD19+ cyCD79a+) , T-ALL (cyCD3+ smCD3lo CD7+), AML (cyMPO+ or CD7+ cyCD3-), and Mixed phenotype (smCD3+ or cyCD3+, CD7+ or CD19+, CD79a+) with the expression of myeloid markers (CD13, CD33, MPO, CD36). The sample acquisition was performed using BD FACSCanto II™ or BD FACSLyric™ cytometers. The flow cytometry data analysis was also performed using the Infinicyt 2.0 software.

The first-line treatment protocol, Total Therapy XV of St. Jude Children's Research Hospital, was documented in accordance with the operational guidelines of the ONCOCREAN.

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The cases treated with the Memphis or Interfan protocols were also recorded. Moreover, the times of diagnosis and disease recurrence within the first 18 months were recorded because this kind of very early relapse was associated with a bad prognosis. Generally, relapse is defined as the return of a disease or its signs and symptoms after a period of improvement. Also, DFS is defined as the disease-free time in months calculated by the difference between the time of diagnosis and relapse. The OS time up to 18 months was recorded too.

Statistical analysis

The quantitative variables were analyzed with the Kolmogorov-Smirnov normality test and showed a free distribution. They were reported as median and interquartile ranges. The statistical comparisons were also made with the Mann-Whitney U test. The qualitative variables were reported in numbers and percentages, using the chi-square or Fisher's exact test to make statistical comparisons. To analyze the probability of an event, an odds ratio was used as an association measurement. Survival curves were plotted with the Kaplan Meier method. The Breslow test was used to compare the groups for both OS and DFS, i.e., for the patients with and without aberrant immunophenotype and antigenic co-expression. The Cox regression analysis was done to determine the variables associated with mortality and relapse up to 18 months of follow-up. Statistical significance was considered to be a p value of < 0.05. The statistical package SPSS V.29.0 (IBM Corp.) served to perform the analyses.

The post-hoc power obtained through OR was calculated using the values from the patients with co-expression of CD66c and CD123; this was associated with relapse. For the patients in the relapse and remission groups, the obtained value was 1.236, with a statistical power of 85%.

Ethical approval

Following what is stated in Article 17 of the General Health Law on Health Research Regulations of Mexico, this study was considered to be without risks. Due to the retrospective nature of the study, informed consent letters were not necessary. The protocol was approved by the Health Research Ethics Committee and the Local Health Research Committee of the Subspecialty Medical Unit, Hospital of Gynecology and Pediatrics # 48, at the Mexican Institute of Social Security under registration number R-2023-1002-017.

Results

A total of 156 children with ALL were included, of whom 67 were female and 89 were male. The mean age of the disease onset was 6.5 years (IQR 4-11), and the age range of the subjects was from 11 months to 17 years. Only one patient developed the disease before turning one year. The basic clinical characteristics of the children at the onset of the disease are shown in Table I.

The patients who expressed positive aberrant markers (PAM) and had a high clinical risk at diagnosis ran a risk of mortality (OR = 5.25, 95% CI 1.6-17.0). All the patients with these characteristics presented relapse. Those with PAM and positive MRD at the end of the induction therapy showed a risk of mortality (OR = 3.6, 95% CI 1.03-12.4) and relapse (OR = 19.8, 95% CI 4.4- 88.8). Negative aberrant markers (NAM) and positive MRD had a risk of mortality (OR = 5.5, 95% CI 1.09-28.18) and relapse (OR = 7, 95% CI 1.2-38.9). These results are presented in Table II.

The number of the aberrant antigens expressed for either death or relapse in patients was around one and four, respectively. Regarding the expression of two or more aberrant antigens in the same patient, the most common antigen co-expression was CD66c and CD123, both for the patients who died (n = 6, 14%) and for those who relapsed (n = 5, 19%) (Table III).

The five most frequent aberrant antigens were compared in terms of distribution. For the relapse risk, the aberrant expression of CD123 showed OR = 4.4, 95% CI 1.5-13.0, while the co-expression of CD123 and CD66c displayed OR = 4.1, 95% CI 1.2-14.4 (Table IV).

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At a mean follow-up time of 15.5 months, with a range of 14.7 to 16.3 months, 41 (26.2%) children with ALL died, and 26 children (16.6%) experienced relapses. The censored data at the estimated follow-up time did not allow for the assessment of the mean survival. This is because the mean exceeded the survival time value of the group studies. However, the OS at a median follow-up time of 11 months was 85% (Figure 1A), and there was no difference between OS (p = 0.390) and DFS (p = 0.220) in the PAM (Figure 1B).

The co-expression of CD66c and CD123 (n = 12) was linked to a shortened OS (p = 0.040) and DFS (p = 0.003), as compared with the rest of the patients who did not express this co-expression (n = 150) (Figure 1C).

To further verify the association of the clinical factors between mortality and relapse, univariate Cox regression analyses were performed.

During 18 months of follow-up, the mortality of the patients with ALL was associated with a leukocyte count of ≥ 50 x 109/L, high risk stratification, positive MRD, and (in the cases where it was expressed) the co-expression of CD66c and CD123. Likewise, high-risk stratification, positive MRD and a single expression of CD123 were associated with a higher risk of relapse for DFS (Table V).

The multivariate Cox regression analysis conducted through the Enter selection method showed that risk factors such as high-risk stratification and positive MRD were associated with shortened OS and DFS (Table VI).

Table I: Clinical and laboratory features of patients with ALL

Characteristics	**Total Cohort N = 156**	**Group I Positive aberrant markers N = 87**	**Group II Negative aberrant markers N = 69**	**P-value**
Age (years) No. (%) < 10 ≥ 10	 101(65) 55(35)	 56(64) 31(36)	 45(65) 24(35	1.000*
Sex No. (%) Female Male	 67 (43) 89 (57)	 42 (48) 45 (52)	 25(36) 44(64)	0.130
WBC No. (%) < 50 x10^9/L ≥ 50 x10^9/L	 126(80) 30(20)	 71(82) 16(18)	 55(80) 14(20)	0.920*
Prednisone response No. (%) PGR PPR	 144(92) 12(8)	 83(95) 4(5)	 61(88) 8(12)	0.100
Risk stratification No. (%) Standard High	 65(41) 91(59)	 35(40) 52(60)	 30(43) 39(57)	0.680
Treatment protocol No. (%) Total XV Other	 147(94) 9(6)	 82(94) 5(6)	 65(94) 4(6)	1.00**
MRD No. (%) Positive Negative	 18(13) 138(87)	 76(87) 11(13)	 62(90) 7(10)	0.570
Immunophenotype No. (%) Pro-B Common B Pre-B Mature B T	 5(3) 11(7) 129(83) 2(1) 9(6)	 4(4) 5(6) 71(82) 1(1) 6(7)	 1(1) 6(8) 58(86) 1(1) 3(4)	0.700

Abbreviations: ALL (acute lymphoblastic leukemia), WBC (white blood cells), PPR (prednisone poor response), MRD (measurable residual disease), *Yates Continuity Correction, **Fisher Exact Probability Test

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Table II: Clinical characteristics of the patients with mortality and relapse according to PAM or NAM

Characteristic	PAM n=87			NAM n=69			PAM n=87			NAM n=69		
	Dead n=25	Live n=62	p	Dead n=16	Live n=53	p	Relapse n=17	Remission n=70	p	Relapse n=9	Remission n=60	p
Age (year) No. (%) > 10	10(40)	21(34)	0.58[a]	7(44)	17(32)	0.39[a]	9(53)	22(31)	0.09[a]	4(44)	20(33)	0.700[b]
Sex No. (%) Male	17(68)	28(45)	0.05[a]	11(69)	33(62)	0.63[a]	12(71)	33(47)	0.08[a]	5(56)	39(65)	0.710[b]
WBC No. (%) $\geq 50 \times 10^9$/L	7(28)	9(15)	0.21[b]	4(25)	10(19)	0.72[b]	6(35)	10(14)	0.07[b]	4(44)	10(17)	0.070[b]
Prednisone response No. (%) PPR	2(8)	2(3)	0.57[b]	2(12)	6(11)	1.0[b]	0(0)	4(6)	0.58[b]	1(11)	7(12)	1.00[b]
Risk stratification No. (%) High	21(84)	31(50)	0.003[a]	12(75)	27(51)	0.08[a]	17(100)	35(50)	<0.001[a]	6(67)	33(55)	0.720[b]
MRD No. (%) Positive	6(27)	5(8)	0.04[a]	4(25)	3(6)	0.04[b]	8(47)	3(5)	<0.001[b]	3(33)	4(7)	0.040[b]

Abbreviations: PAM (positive aberrant markers), NAM (negative aberrant markers), WBC (white blood cells), PPR (prednisone poor response), MRD (measurable residual disease),
[a] Person´s Chi-square test, [b] Fisher Exact Probability Test

Table III: Antigens expressed in the patients who died and those with relapse with aberrant immunophenotype 18 months after the diagnosis

Antigens No. (%)	Dead N = 25	Relapse N = 17
CD66c	13(32)	10(39)
CD9	7(17)	5(19)
CD123	7(17)	7(27)
CD24	4(10)	1(4)
CD58	2(5)	0(0)
CD33	2(5)	1(4)
CD117	2(5)	1(4)
CD79	1(2)	0(0)
CD16	0(0)	1(4)
CD10	1(2)	0(0)
CD81	1(2)	0(0)
CD97	1(2)	0(0)

CD, Cluster of differentiation
The number of antigen expressions was variable, from one to four in the same child.

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Table IV: Aberrant antigen expression in the ALL patients who died or relapsed

Antigen No. (%)	Dead N = 41	Live N = 115	*p*	Relapse N = 26	Remission N = 130	*p*
CD66c Positive	13(32)	30(26)	0.48 [a]	10(39)	33(25)	0.170 [a]
CD9 Positive	7(17)	21(18)	0.86 [a]	5(19)	23(18)	1.00 [b]
CD24 Positive	4(10)	21(18)	0.20 [a]	1(4)	24(18)	0.110 [b]
CD123 Positive	7(17)	10(9)	0.15 [b]	7(27)	10(8)	0.010 [b]
CD58 Positive	2(5)	12(10)	0.35 [b]	0(0)	14(11)	----

Abbreviations: ALL (acute lymphoblastic leukemia), CD (cluster differentiation)
a Person´s Chi-square test, b Fisher Exact Probability Test

Table V: Univariate Cox regression analyses for risk factors associated with OS and DFS

	OS				**DFS**			
Characteristics	**Wald**	**HR**	**95% CI**	***p***	**Wald**	**HR**	**95% CI**	***p***
Age (years)								
< 10		1.00				1.00		
> 10	1.03	1.38	0.741-2.570	0.30	1.00	1.51	0.673-3.390	0.310
Sex								
Female		1.00				1.00		
Male	2.49	1.69	0.880-3.281	0.11	2.49	1.69	0.880-3.281	0.110
WBC x10^9/L								
< 50		1.00				1.00		
≥ 50	5.688	2.61	1.187-5.774	0.01	1.57	1.55	0.780-3.107	0.210
Prednisone response								
PGR		1.00				1.00		
PPR	0.315	1.34	0.479-3.371	0.57	0.53	0.47	0.064-3.497	0.460
Risk stratification								
Standard		1.00				1.00		
High	9.515	6.65	1.995-22.164	0.002	9.55	3.38	1.563-7.333	0.001
MRD								
Negative		1.00				1.00		
Positive	32.27	9.89	4.486-21.808	< 0.001	10.28	3.26	1.584-6.737	0.001
Aberrant								
Negative		1.00				1.00		
Positive	0.64	1.29	0.691-2.423	0.42	1.32	1.60	0.717-3.609	0.240
CD66c								
Negative		1.00				1.00		
Positive	0.42	1.24	0.644-2.400	0.51	1.88	1.73	0.789-3.835	0.170
CD9								
Negative		1.00				1.00		
Positive	0.03	0.92	0.409-2.082	0.84	0.00	1.04	0.393-2.763	0.930
CD24								
Negative		1.00				1.00		
Positive	1.228	0.55	0.199-1.566	0.26	2.54	0.19	0.027-1.450	0.110
CD 123								
Negative		1.00				1.00		
Positive	2.34	1.88	0.837-4.263	0.12	10.66	4.27	1.787-10.204	0.001
CD66c and CD123								
Negative		1.00				1.00		
Positive	3.797	2.36	0.995-5.632	0.05	8.01	4.13	1.548-11.059	0.005

Abbreviations: OS (overall survival), DFS (disease free survival), HR (hazard ratio), CI (confidence interval), WBC (white blood cell), PGR (prednisone good response), PPR (prednisone poor response), MRD (measurable residual disease), CD (cluster differentiation)

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Table VI: Multivariate Cox regression analyses of the risk factors associated with OS and DFS

	OS				DFS			
	Wald	**HR**	**95% CI**	***p***	**Wald**	**HR**	**95% CI**	***p***
Risk stratification								
Standard		1.00				1.00	1.616-18.625	
High	7.92	3.31	1.439-7.619	0.005	7.45	5.48		0.006
MRD								
Negative		1.00				1.00		
Positive	6.69	2.72	1.275-5.819	0.010	26.62	8.59	3.795-19.446	< 0.001
CD 123								
Negative						1.00		
Positive					3.62	2.42	0.974-6.056	0.050
CD66c+CD123								
Negative						1.00		
Positive					0.60	1.44	0.573-3.638	0.430

Abbreviations: OS (overall survival), DFS (disease free survival), HR (hazard ratio), CI (confidence interval), MRD (measurable residual disease), CD (cluster differentiation)

Figure 1A

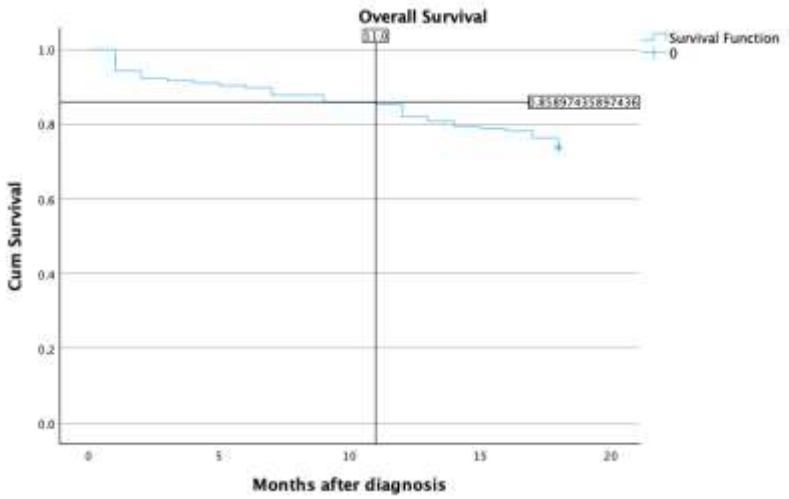

Figure 1B

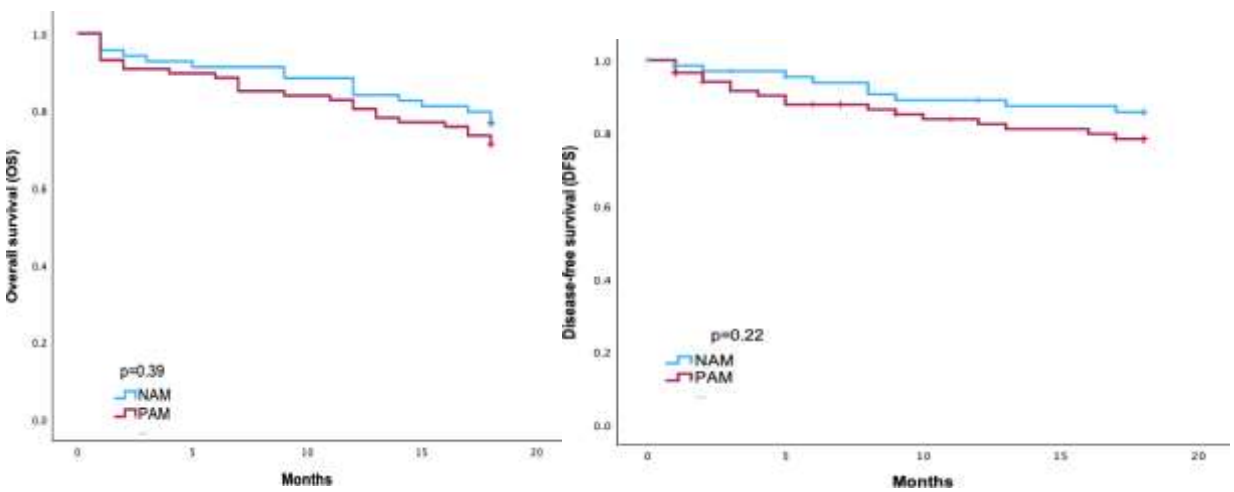

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Figure 1C

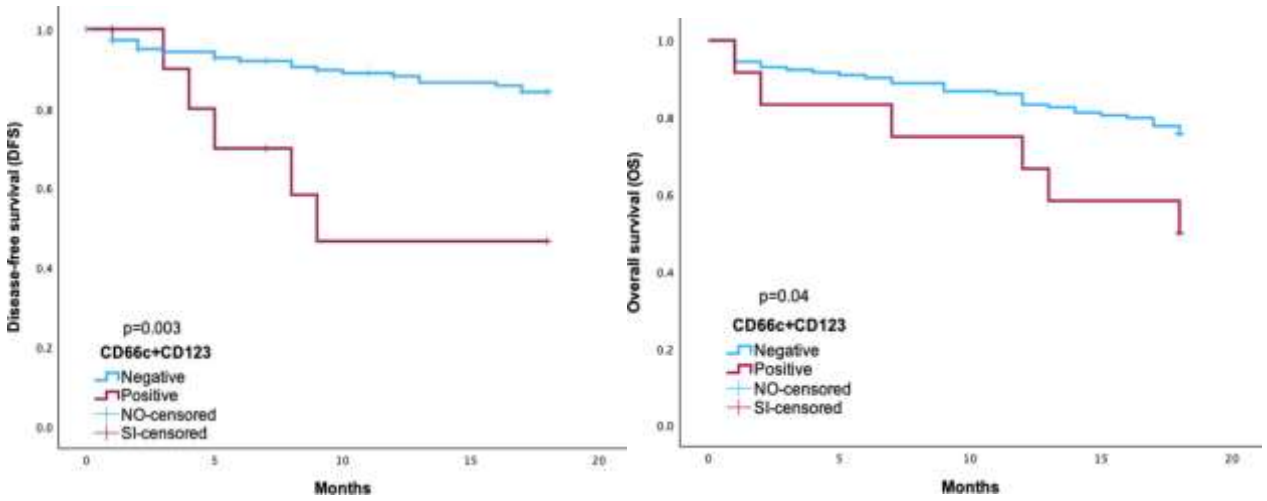

Figure 1 A. Median of OS at 18 months of follow up.
Figure 1 B. Comparison of OS and DFS curves based on aberrant immunophenotype, assessed by the Kaplan-Meier method.
Figure1 C. Comparison of OS and DFS curves based on the CD66c + CD123 antigen combination (n=12), assessed by the Kaplan-Meier method.

Discussion

In pediatric patients with ALL, OS, and DFS up to 18 months of follow-up from diagnosis were similar in cases with PAM and NAM. However, the co-expression of the aberrant antigens CD66c and CD123 had a higher frequency of death and relapse.

Relapse is still the most common cause of treatment failure in about 15-20% of children with ALL. Very early relapses occur in 25% of cases, and the second remission is challenging. Some studies have reported 5-year OS with chemotherapy to be less than 5% of cases and with hematopoietic stem cell transplantation to be 25% (6). Another study has linked relapse of ALL to T lineage with OS of 25.8% (7). Very early relapse has also been characterized as a determinant if induction fails in the second remission (9). In this study, throughout the first 18 months, very early relapse occurred in 26% of the cases. Another study with a similar population reported very early relapse in 39.4% of the cases, with OS and DFS below 60% at the age of three, just as outlined in the rest of Latin America (10-12).

In addition, as seen with other authors (13-15), positive MRD and high-risk stratification were found to be the factors associated with OS and DFS. Clinical and biological characteristics at the onset of the disease are considered key for predicting the disease behavior and classifying patients as early or late responders, directly impacting DFS, especially in the first years after diagnosis and treatment.

The antigen expression in leukemia may vary depending on the geographical location. The behavior of the disease changes among different ethnic or racial groups. In this investigation, 56% of the cases with ALL expressed at least one aberrant antigen. The expressed antigens with a higher frequency in this study differ from those reported in other countries (16, 17).

The impact of aberrant antigens on B-cell ALL was analyzed in pediatric patients with co-expression of CD66c and CD123. A higher proportion of relapses was observed during the 18 months of follow-up, which was probably associated with the BCR-ABL fusion gene (18-20). The CD66c antigen is involved with the adhesion of other surface glycoproteins, such as e-selectin, as well as with migration, signal transduction and gene expression regulation. The existence of CD66c is limited to granulocytes and their precursors, while

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aberrant expression in B-cell lineage blasts is associated with the Philadelphia chromosome and hyperdiploidy (21). In the present study, the isolated aberrant expression of this antigen did not show a statistically significant difference between the clinical variables or for the outcome of death or relapse.

In a Japanese study where children with B-cell ALL were studied, it was found that the single expression of CD66c in pediatric ALL had no impact on prognosis and was not specific to the presence of BCR-ABL. However, genetic abnormalities per se of CD66c, such as hyperdiploidy and CRLF2, were considered as important prognostic factors. Clinical implications of CD66c are not limited to its single expression, since it becomes relevant when associated with other antigens. This happens particularly in genetic abnormalities identified with BCR-ABL and Philadelphia-like ALL (22).

The CD123 antigen, also known as interleukin 3-alpha, is a molecule expressed in plasmacytoid dendritic cells. The overexpression of this antigen in B lymphoid blasts is associated with hyperdiploid karyotypes (23) and expression of the BCR-ABL1 fusion gene, the latter exhibiting increased expression in relapses rather than at the moment of diagnosis (24). In the present study, the expression of CD123 was more frequent in the patients who had a relapse in the first 18 months after diagnosis than in those who died in the same period of time. In this research, the recorded events of death or relapse did not reach the mean survival because the censored events exceeded the total follow-up time. Despite this finding, the results allow for the identification of the population-related characteristics that confer high clinical risk at the moment of diagnosis, disease-related characteristics like MRD+ at the end of the induction therapy and co-expression of CD66c and CD123 antigens. These were related to very early relapse, which is considered as the worst case scenario to achieve a second remission. It is important to mention that relapses occur in up to 30% of patients with ALL, this being the main cause of shortened OS. They have been related to higher intensity treatment regimens that imply greater morbidity and, in some cases, have led to death.

In this study, the co-expression of CD66c and CD123 antigens seemed relevant for OS and DFS in pediatric patients with ALL. This association was only present in 13.8% of the patients who expressed aberrant immunophenotype. The disease outcomes are based on the time at which relapse occurs, the cellular lineage and clinical characteristics present at its debut, as described in the determinants of survival after the first relapse event by the Children's Oncology Group (COG) (8). The CD66c and CD123 co-expression subgroup is small, hence the data should be considered exploratory. This requires more studies to confirm the results.

There is a limited amount of data available about the co-expression of CD66c and CD123. The present results may contribute to providing an enhanced MRD follow-up in patients who have this co-expression. It is discouraging when patients present a relapse in the first 18 months, since it is likely that they will not have an adequate response to the following treatment and not reach a second remission; even more so when they exhibit co-expression of CD66c and CD123 antigen. The clinical behavior of this co-expression may differ by region and ethnicity; therefore, genetic testing becomes indispensable for clearing up the way and improving the results.

The factors that intervened in OS all through 18 months after diagnosis were WBC count, risk stratification at diagnosis and MRD. For DFS, the same factors were found except for WBC count. These findings are like those reported in different countries on the factors associated with survival (9, 25, 26).

In the present study, positive MRD stood out as the most important independent factor associated with relapse. It is known about the importance that MRD has over OS and DFS in patients with ALL, as stated by the COG (27). The patients with high clinical risks and positive MRD at the end of induction therapy, for whom molecular biology is not always

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available, may benefit from determining MRD at later stages during treatment follow-up. This allow for timely decision making.

The role of ethnicity and race in ALL behavior has been studied too. In the comparison of survival between Caucasians and Latinos, the survival was shown to be lower for the latter (28, 29). There are also reports about environmental risk factors, disparities in socioeconomic status, genetic mutations, and the combination of these factors. An increase in the incidence of ALL was reported in Latinos from 1992 to 2011 (30).

In relation to Mexico City, during the last 10 years, the frequency of ALL was among the highest in the world with a standardized average annual incidence rate of 60 cases per million children (31, 32). The Latino population is characterized by the presence of genetic variants that are linked to their ancestors, with a line of inheritance between Indigenous and European people. This may be the reason for the appearance of some mutations associated with the Philadelphia-like chromosomes in this population, which, by itself, leads to a poor prognosis (33).

The main limitation of the study was the lack of specialized tests, like cytogenetics, for a better diagnostic precision of the mutations present in the studied pediatric population. However, this study contributes valuable data from the Mexican population, for which there is limited research on pediatric ALL. The finding on CD66c and CD123 co-expression that predicts worse outcomes is novel and clinically relevant. Furthermore, it is proposed that future research analyze molecular biology reports, at least about the most frequent transcripts in ALL. This would help to know the expression pattern of antigens.

Another limitation of the study was that the patients received different treatment schedules. Nevertheless, this was not considered a variable for the multivariate analysis because a minority of the participants received a different treatment regimen.

Regarding the positive MRD reports, knowing the antigens that remained at the end of the induction therapy and their percentage of positivity, in relation to the probability of relapses, could help with the decision making of a timely treatment. As mentioned above, the goal was to investigate the behavior of the disease during the first 18 months after diagnosis, due to its high impact on survival after the relapse event.

Focusing on the strengths of this study, it was possible to identify the immunophenotypic characteristics of ALL in the Bajío region of Mexico, which differs from what was reported by North American and European studies. As it was observed, the co-expression of CD66c with CD123 influenced both OS and DFS, while there was an association with an increased relapse risk. The individual expression of CD123 was associated with positive MRD and an increased risk of relapse. The earlier results were only significant in the univariate analysis, losing their statistical significance in the multivariate analysis. This study confirmed that both MRD and risk stratification at diagnosis are the cornerstone outcome of the disease. It is also worth noting that this study was carried out exclusively in a pediatric population. It is suggested to increase the follow-up time in future studies so as to monitor the behavior of CD66c and CD123 co-expression on the disease evolution, at least for three years.

Conclusion

In the univariate analysis, CD66c and CD123 co-expression was associated with decreased OS and DFS and a higher risk of relapse. However, this association did not remain significant in the multivariate model, indicating that it is not an independent prognostic factor. In contrast, isolated CD123 expression was linked to positive MRD and an increased risk of relapse. These observations provide clinically relevant preliminary insights, particularly in settings with survival disparities, but they require confirmation in larger cohorts with extended follow-up and integrated molecular data.

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Availability of Data

The data that support the findings of this study are available on request from the corresponding author, Gloria Patricia Sosa-Bustamante. The data are not publicly available due to privacy restrictions, since the data contains information that could compromise the privacy of research participants.

Ethical Considerations

Following what is written in the Article 17 of the General Health Law on Health Research Regulations of Mexico, this study was considered as of without risk. Due to the retrospective nature of this study, informed consent letters were not necessary. The protocol was approved by the Health Research Ethics Committee and the Local Health Research Committee of the Subspecialty Medical Unit, Hospital of Gynecology and Pediatrics # 48, Mexican Institute of Social Security under registration number R-2023-1002-017.

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Authors' Contributions

Betzayda Valdez-Garibay was in charge of writing, review and editing of the original draft, supervision, methodology, investigation, formal analysis, and conceptualization. Carlos Paque-Bautista, Benigno Linares-Segovia, Alma Patricia González and Octavio Martínez-Villegas undertook conceptualization, methodology, validation, formal analysis, investigation, writing, review, editing, visualization, and supervision. Dalia Ramírez-Ramírez and Rosana Pelayo did supervision, project administration, investigation, and data curation. Gloria Patricia Sosa-Bustamante performed conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing the original draft, –review, editing, visualization, supervision, and project administration.

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Conflict of Interest

The authors declare no conflict of interests regarding this study.

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