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Evaluation of the Correlation of Immunohistochemical Findings with Flow Cytometric Findings in Newly Diagnosed Pediatric Acute Lymphoblastic Leukemia Patients

Yeni Tanı Pediatrik Akut Lenfoblastik Lösemide, Kemik İliği Biyopsisinde İmmünohistokimyasal Bulguların Akım Sitometrik Bulgular ile İlişkisinin Değerlendirilmesi

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Abstract

Aim: The development of new therapeutic options to treat leukemia (therapies targeting chimeric antigen receptor (CAR) T cells) down-regulates markers expressed on the cell surface. Therefore, conventional immunophenotyping panels no longer make these antigens unreliable for identifying a B cell immunophenotype. In our study, we methodically compared multiparametric flow cytometry (FC) in bone marrow aspiration and immunohistochemical (IHC) analysis in bone marrow biopsy in childhood acute lymphoblastic leukemia (ALL). We sought to answer whether these two methods could be alternatives to each other in the diagnosis of leukemia.

Material and Method: Twenty-eight patients diagnosed with ALL were included in the study. A Kappa test was performed between the expression rates of the antibodies studied in simultaneous FC and IHC studies in bone marrow aspiration and biopsy samples performed at the initial diagnosis.

Results: Twenty-three of the patients were precursor B-ALL (BCP-ALL) and 5 were T-ALL. In the immunophenotyping of patients with BCP-ALL using FC and IHC, MPO, CD79A, CD14, CD3 expressions were the same, while CD19, CD7, CD117, CD33, CD 56, CD34 expressions were very good, good concordance for CD20 expressions and moderate for CD10 expressions. In immunophenotyping of patients diagnosed with T-ALL using FC and IHC, CD20, CD19, CD14, CD79a, MPO, CD22 expressions were the same and excellent agreement was found in terms of CD2, CD10, CD34 expressions.

Conclusion: In cases where there are treatments that affect immunophenotyping, costly methods such as FC are not available, or bone marrow aspiration cannot be taken adequately, immunophenotyping with IHC can be safely performed in the diagnosis of pediatric ALL in bone marrow biopsy.

Keywords: Acute Lymphoblastic leukemia, immunohistochemical, flow cytometry, immunophenotyping

Öz

Amaç: Lösemiyi tedavi etmek için yeni terapötik seçeneklerin geliştirilmesi (kimerik antijen reseptörü (CAR) T hücrelerini hedefleyen tedaviler), hücre yüzeyinde eksprese edilen belirteçlerin down regülasyonuna neden olmaktadır. Bu nedenle, geleneksel immünofenotipleme panelleri artık bu antijenleri bir B hücresi immünofenotipinin tanımlanması için güvenilmez hale getirmektedir. Çalışmamızda, çocukluk çağı akut lenfoblastik lösemisinde (ALL) kemik iliği aspirasyonunda multiparametrik akım sitometrisi (AS) ile kemik iliği biyopsisinde immünohistokimyasal (İHK) analizi metodolojik olarak karşılaştırdık. Lösemi tanısında bu iki yöntemin birbirine alternatif olup olamayacağını yanıtlamaya çalıştık.

Gereç ve Yöntem: ALL tanısı alan 28 hasta çalışmaya dahil edildi. İlk tanı sırasında yapılan kemik iliği aspirasyonu ve biyopsi örneklerinde eş zamanlı AS ve İHK çalışmalarında kullanılan antikorların ekspresyonları arasındaki uyum için Kappa testi yapıldı.

Bulgular: Hastaların 23'ü pre-B ALL (BCP-ALL) ve 5'i T-ALL idi. BCP-ALL'li hastaların AS ve İHK'da kullanılan MPO, CD79A, CD14, CD3 antikorlarında ekspresyonlar aynı iken, CD19, CD7, CD117, CD33, CD 56, CD34 antikor ekspresyonları arasında çok iyi, CD20 antikor ekspresyonunda iyi ve CD10 ekspresyonunda ise orta düzeyde uyum mevcuttu. T-ALL tanılı hastaların AS ve İHK'da kullanılan CD20, CD19, CD14, CD79a, MPO, CD22 antikorlarının ekspresyonları aynıydı, CD2, CD10, CD34 antikorlarının ekspresyonları açısından çok iyi uyum mevcuttu.

Sonuç: İmmünfenotiplemeyi etkileyen tedavilerin olduğu, AS gibi maliyetli yöntemlerin bulunmadığı veya kemik iliği aspirasyonunun yeterince alınamadığı durumlarda, kemik iliği biyopsisinde pediatrik ALL tanısında İHK ile immünfenotipleme güvenle yapılabilir.

Anahtar Kelimeler: Akut lenfoblastik lösemi, immünohistokimya, akım sitometri, immünofenotiplendirme

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer and constitutes approximately 25% of cancer diagnoses among children under the age of 15.[1-3] ALL is the most successful treatment paradigm in pediatric cancer medicine as illustrated by the significant survival rate. İmprovement from ~10% in the 1960s to >90% today.[4] Despite high treatment rates, it is an important cause of mortality and morbidity.[5] ALL originates from B-cell precursors (BCP-ALL) in the bone marrow (BM) and T-cell precursors (T-ALL) in the bone marrow and thymus.[1] BCP-ALL constitutes the majority of childhood leukemias, while T-ALL constitutes less. T-ALL incidence increased in adolescents.[6] The incidence of ALL in children varies by country and ranges from 2.5 to 4.1/100.000.[7] Classification of leukemias has changed over time,[8] but the latest classification includes morphological, immunophenotypic, and identification of genetic aberrations evaluations.[6] After acute leukemias can quickly spread to other parts of the body, particularly the spleen, lymph nodes, liver, and brain.[9] For the diagnosis of acute leukemia, the blast rate in the BM should be above 20%.[6] BM biopsy and aspiration are essential for the diagnosis and treatment of leukemia. Classification of leukemia should be done for treatment. Immunophenotyping is one of the most important parameters in ALL classification.[6] For this, multiparametric flow cytometry (FC)[10] and immunohistochemical study (IHC) in BM biopsy are used as methods.[11] Multiparametric FC has important advantages in terms of rapid results and its use in the detection of minimal residual disease,[12] but it also has challenges in the evaluation such as low cell volume, low cell viability, and increased data.[13] In the presence of fibrosis in the BM, the amount of cells taken for FC is considerably reduced, and sometimes it prevents the diagnosis with FC, and BM biopsy becomes the most important tool in the diagnosis.[14]

Even if they seem rare, in biphenotypic leukemias with diagnostic difficulties, especially if MPO positivity cannot be detected in FC, an IHC study in biopsy may be required for diagnosis.[6] Current immunotherapy models (CD 19 or CD22 targeting chimeric antigen receptor (CAR) T cells, blinatumomab, inotuzumab) downregulate cell surface-expressed markers.[15] Therefore, conventional immunophenotyping panels can no longer rely on these antigens to define a B cell immunophenotype. Therefore, there is a need for alternative immunophenotyping besides creating new panels.

Our aim in this study is to compare immunophenotyping with FC in pediatric ALL cases and with IHC in bone marrow biopsy. To find alternative methods in immunophenotyping.

MATERIAL AND METHOD

Twenty-eight patients diagnosed with ALL between 2017-2021 were included. The diagnosis of ALL was made by peripheral smear, BM aspirate, BM biopsy, FC analysis, and IHC studies performed on biopsies. ALL cases were divided into two groups as BCP-ALL and T ALL. BM biopsies were subjected to routine tissue procedures after 10% formaldehyde fixation, decalcified using 14% EDTA solution for 24 hours were embedded in paraffin. Four-micron deparaffinized tissue sections were routinely stained with hematoxylin eosin (H&E). For each antibody in the study, four micron-thick sections from the tissues in the paraffin blocks were included in the research, and we took the control group on poly -L-lysine coated slides. In the IHC studies, the antigen retrieval technique was used, and the avidin-biotin-peroxidase complex method was applied. The antibodies were examined in a Leica band max automatic immunohistochemical device. The Bond Polymer Refine Detection kit (Leica, DS9800) was used for each antibody. The necessary staining procedure on the data sheet was applied, and appropriate positive and negative controls were used for each antibody. For most antibodies, normal cells from bone marrow were used as positive and negative controls, endothelial cells for CD34 (**Figure 1**-**2**), thymus tissue for tdt. The characteristics of the primary anticipation used in the immunohistochemical study are listed (**Table 1**). The prepared samples were examined under an Olympus BX51 model microscope after the coverslip was covered with the ultra-mount.

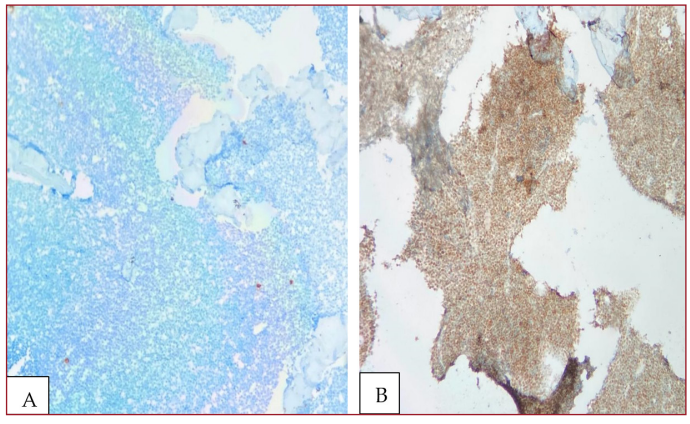

Figure 1. While there is a negative reaction with MPO antibody in leukemia cells, cytoplasmic positive reaction is observed in cells belonging to myeloid series. Positive myeloid cells were considered as internal control (A, x100). While there is nuclear reaction with PAX-5 antibody in leukemia cells, more severe nuclear reaction is observed in normal B lymphocytes other than leukemia cells (B, x100).

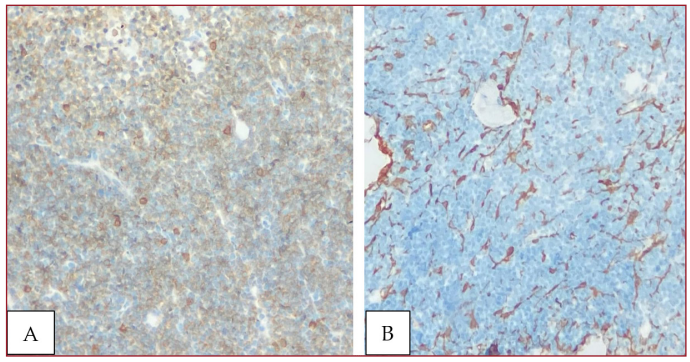

Figure 2. While cytoplasmic reaction is present with CD8 antibody in leukemia cells, more severe cytoplasmic reaction is observed in normal T lymphocytes other than leukemia cells (A,x100). While there is a positive cytoplasmic reaction in vascular endothelium with CD34 antibody, a negative reaction is observed in leukemia cells (B,x100).

Table 1. Antibodies used in immunohistochemical study and their properties.

Antibody	Clone	Dilution	Incubation Time	Antigen retrieval	Company
MPO	Polyclonal	1:200	5minute	ER1	Thermo
CD3	LN10	1:300	40minute	ER2	Leica
CD19	ZR212	1:100	30minute	ER2	Zeta
CD14	7	1:100	30minute	ER1	Bias
TdT	SEN28	1:50	30minute	ER2	Leica
CD34	ABEND/10	1:200	20minute	ER1	Leica
CD117	EP10	1:200	20minute	ER2	Leica
CD22	SP104	1:50	20minute	ER1	Zeta
CD10	56C6	1:100	40 minute	ER2	Leica
CD20	L26	1:200	40 minute	ER2	Leica
CD79A	HM47/A9	1:150	40 minute	ER2	Thermo
PAX5	Polyclonal	1:80	40 minute	ER2	Thermo
CD56	CD564	1:100	30minute	ER1	Leica
CD33	PWS44	1:100	30minute	ER2	Leica
CD4	EP204	1:100	20minute	ER2	Epitomics
CD8	4B11	1:50	30minute	ER2	Leica
CD1A	O10	1:70	30minute	ER2	Thermo
CD99	EPR3097Y	1:50	40 minute	ER2	Biocare
CD5	4CY	1:100	20minute	ER2	Leica
CD13	304	1:80	30minute	ER2	Novocastra
CD123	BR4MS	1:25	30minute	ER2	Leica

ER1: Citrate buffer ; ER2: EDTA buffer.

Staining in 10% and more of leukemia cells was considered positive for each antibody in the immunohistochemical evaluation.[16-18]

In the FC study, the analyses on the BM samples taken into tubes containing Ethylenediaminetetraacetic acid (EDTA) were completed within 24 hours. CD45, CD19, CD20, CD79a, CD22, CD3, CD5, CD123, CD38, CD10, CD7, CD79a, CD34, CD117, CD14, CD33, MPO, CD13, CD33, HLA-DR antibodies were used in the BCP-ALL immunophenotyping panel and CD45, surface CD3, cytoplasmic CD3, CD2, CD7, CD5, CD4, CD8, CD10, CD38, CD19, CD123, CD34, CD117, CD99, CD1A, TDT, CD33, CD14, CD20, CD22, CD56 antibodies were used in the T-ALL immunophenotyping panel. The reading results of the samples obtained were evaluated after the application of antibodies and the lysing procedure. Readings were performed on the Navios Ex model device of Beckman Coulter, (Miami, USA) using antibodies from the same company (**Table 2**). First, gating was performed on the CD45-Side Scatter (SSC) graph. In MPO FC evaluation, 10% was accepted for cut-off expression and 20% cut-off was accepted for other antibodies (**Figure 3**-**4**).[19-21] For each antibody, positive and negative expressions in normal cells were used as controls whenever possible. During our applications, the cut-off values created for our laboratory were taken into consideration.

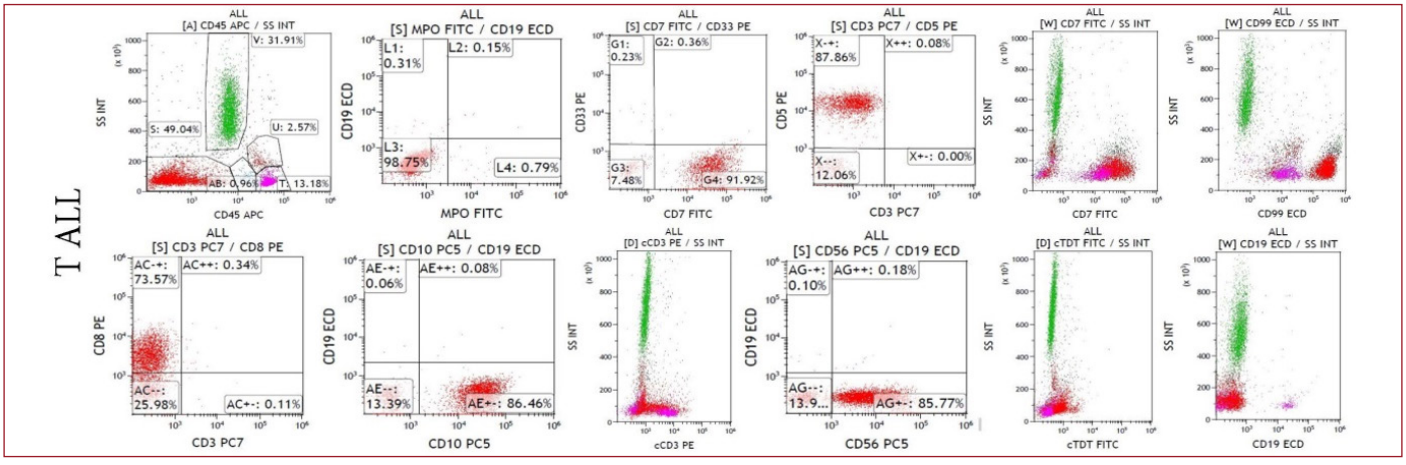

Figure 4. Leukemia cells are negative with CD45, Tdt, CD3, MPO, CD19 antibodies and positive with CD5, CD7, CD8, CD10, CD56, cCD3 antibodies. Myelocyte cells are used as internal control for MPO, and lymphocytes as internal control for both T cell markers and B cell markers.

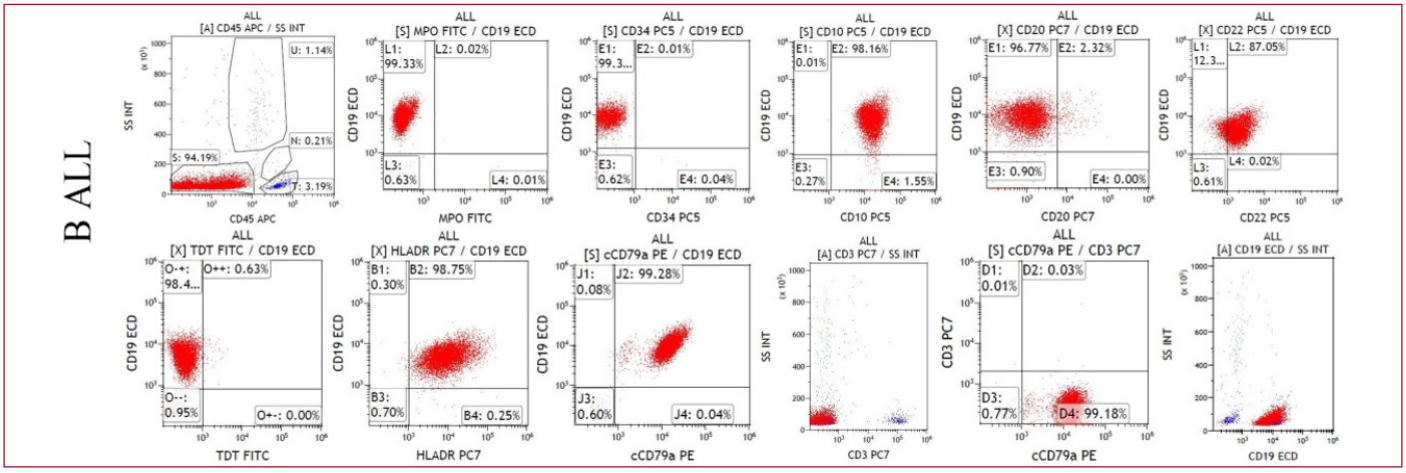

Figure 3. Leukemia cells are negative with CD45, Tdt, CD3, MPO antibodies, and positive with CD19, CD10, CD79A antibodies. Myelocyte cells are used as internal control for MPO antibody and lymphocytes are used as internal control for both T cell markers and B cell markers.

Table 2. Antibodies used in flow cytometry and their characteristics.

BPC-ALL, T-ALL	Antibody	Color	Clone
1	CD1a	PC5	BL6 (IgG1 mouse)
2	CD2	FITC	39C1.5 (IgG2a Rat)
3	CD3	PC7	UCHT1 (IgG1 mouse)
4	CyCD3	PE	UCHT1 (IgG1 mouse)
5	CD4	ECD	SFCL12T4D11(IgG1 mouse)
6	CD5	PE	BL1a (IgG2a mouse)
7	CD7	FITC	8H8.1 (IgG2a mouse)
8	CD8	PE	B9.11 (IgG1 mouse)
9	CD10	PC5	ALB1 (IgG1 mouse)
10	CD11a	PE	25.3 (IgG1 mouse)
11	CD13	PE	SJ1D1 (IgG1 mouse)
12	CD14	PC7	RMO52 (IgG2a mouse)
13	CD19	ECD	J3-119 (IgG1 mouse)
14	CD20	PC7	B9E9 (IgG2a mouse)
15	CD22	PC5	SJ10.1H11 (IgG1 mouse)
16	cyCD22	PC7	SJ10.1H11 (IgG1 mouse)
17	CD33	PE	D3HL60.251 (IgG1 mouse)
18	CD34	PE	581 (IgG1 mouse)
19	CD34	PC5	581 (IgG1 mouse)
20	CD38	PC7	LS198-4-3 (IgG1 mouse)
21	CD45	APC	J33 (IgG1 mouse)
22	CD56	PC5	N901 (IgG1 mouse)
23	CD58	FITC	ALCD58 (IgG2a mouse)
24	CD71	PE	YDJ1.2.2 (IgG1 mouse)
25	CyCD79a	PE	HM47 (IgG1 mouse)
26	CD99	ECD	HCD99 (IgG2a mouse)
27	CD117	ECD	104D2D1 (IgG1 mouse)
28	CD123	PC5,5	SSDCLY107D2 (IgG1 mouse)
29	HLA-DR	PC7	Immu-357 (IgG1 mouse)
30	IgM	FITC	SA-DA4 (IgG1 mouse)
31	CyIgM	PE	SA-DA4 (IgG1 mouse)
32	MPO	FITC	CLB-MPO-1 (IgG2a mouse)
33	TCRalb	PE	1P26A (IgG1 mouse)
34	TCRgld	FITC	IMMU510 (IgG1 mouse)
35	TDT	FITC	HT1+HT4+HT8+HT9 (IgG mouse)

BPC-ALL: B-cell precursors acute lymphoblastic leukemia, T-ALL: T-cell acute lymphoblastic leukemia.

Statistical Method

Continuous variables were presented as median, maximum and minimum, categorical variables were presented as percentage (%) and frequency (n). Kappa statistics were performed for antibodies studied in both BM biopsy and FC. In order for this analysis to be performed, it is necessary to have a 2*2 table layout. Kappa coefficient (kappa value) was not calculated for cases where only one of the negative or positive results was observed. Kappa coefficient varies between -1 and 1; classifies as , perfect agreement (0.81-1), good agreement (0.61-0.80), moderate agreement (0.41-0.60), low agreement (0.21-0.40), weak agreement (0.00-0.20), and very weak agreement (<0.00). If the p value was < 0.05, it was considered significant.[22]

Ethical approval

Ethics The study was approved by the Afyonkarahisar Health Sciences University Clinical Research Ethics Committee (2021/382).

RESULTS

A total of 28 patients, 23 (82.1%) of whom were diagnosed with BCP-ALL and 5 (17.9%) with T-ALL, were included in the study. Of the children diagnosed with BCP-ALL, 16 (69.6%) were male. Of the children diagnosed with T-ALL, 4 (80.0%) were male. The median age of children with BCP-ALL was 7 years (2-17 years) and T-ALL was 8 years (3-14 years).

In BCP-ALL, according to the FC result, CD19 and CD79A were positive in all the cases, while CD22 and CD10 were positive over 90% of the cases. All MPO, CD19 and CD3 and 80% of CD56 and CD117 were negative, while according to the results of IHC in BCP-ALL, positivity was observed in all CD79 A and TST, and over 80% of CD19 and CD10. Negativity was observed in all MPO, CD14, and CD3 and in more than 80% of CD56 and CD117 (**Table 3**).

Table 3. Results of antibodies in BCP-ALL that were studied in all patients with both FC and IHC.

Antibody	Flow-cytometry		Immunohistochemistry	
	+	-	+	-
	n(%)	n(%)	n(%)	n(%)
CD19	23 (100%)	0 (0%)	22 (95.7%)	1 (4.3%)
CD79A	23 (100%)	0 (0%)	23 (100%)	0 (0%)
CD22	21 (91.3%)	2 (8.7%)	18 (78.3%)	5 (21.7%)
CD10	22 (95.7%)	1 (4.3%)	20 (87.0%)	3 (13.0%)
CD20	8 (34.8%)	15 (65.2%)	6 (26.1%)	17 (73.9%)
CD34	19 (82.6%)	4 (17.4%)	18 (78.3%)	5 (21.7%)
CD33	7 (30.4%)	16 (69.6%)	6 (26.1%)	17 (73.9%)
CD117	3 (13.0%)	20 (87.0%)	4 (17.4%)	19 (82.6%)
MPO	0 (0%)	23 (100%)	0 (0%)	23 (100%)
CD14	0 (0%)	23 (100%)	0 (0%)	23 (100%)
CD56	2 (8.7%)	21 (91.3%)	2 (8.7%)	21 (91.3%)
TDT	18 (78.3%)	5 (21.7%)	23 (100%)	0 (0%)
CD3	0 (0%)	23 (100%)	0 (0%)	23 (100%)
PAX5	-	-	23 (100%)	0 (0%)

BPC-ALL: B-cell precursors acute lymphoblastic leukemia. IHC: Immunohistochemistry. FC: Flow Cytometry.

CD123, CD7, CD13 could be applied in different numbers. PAX 5 was performed as IHC only on biopsy and all cases were positive. CD38 and HLA-DR were studied in FC and all cases were positive (**Table 4**).

Table 4. Results of antibodies in BCP-ALL that were studied with both FC and IHC in some patients.

Antibody	Flow-cytometry		Immunohistochemistry	
	+	-	+	-
	n (%)	n (%)	n (%)	n (%)
CD123	2 (22.2)	7 (87.8)	9 (69.2)	4 (30.8)
CD7	1 (4.3%)	22 (95.7%)	1 (10%)	9 (90.0%)
CD13	6 (26.1%)	17 (73.9%)	0 (0%)	8 (100%)

BPC-ALL: B-cell precursors acute lymphoblastic leukemia. IHC: Immunohistochemistry. FC: Flow Cytometry.

In BCP-ALL, as a result of both FC and IHC studies, all MP0, CD3, CD14 results were negative and CD79a CD13 results were all positive. There was substantial agreement between FC and IHC studies for CD19, CD7, CD117, CD33, CD56 (k>0,80)(**Table 5**).

Table 5. Level of agreement of antibodies studied by FC and IHC in BCP-ALL.

Antibody	k	Agreement level	p
CD19	1.000	PERFECT	<0.001
CD123	0.125	WEAK	0.495
CD22	-0.142	VERY WEAK	0.435
CD10	0.465	MODERATE	0.008
CD20	0.796	GOOD	<0.001
CD34	0.862	GOOD	<0.001
CD7	1.000	PERFECT	0.002
CD117	0.832	PERFECT	<0.001
CD33	0.893	PERFECT	<0.001
CD56	1.000	PERFECT	<0.001

BPC-ALL: B-cell precursors acute lymphoblastic leukemia. IHC: Immunohistochemistry. FC: Flow Cytometry.

In T-ALL cases, according to the FC result, CD1A, CD5 and CD7 were found to be positive in all cases. CD117, MPO, CD14, CD22, CD20, CD33, CD79A and CD19 were found to be negative in all cases, and according to the results of IHC in T-ALL, CD3 and CD7 were all positive. CD117, MPO, CD14, CD22, CD20, CD33, CD79A and CD19 were all negative (**Table 6**).

Table 6. Results of antibodies in T-ALL that can be studied in all patients with both FC and IHC.

Antibody	Flow-cytometry		Immunohistochemistry	
	+	-	+	-
	n(%)	n(%)	n(%)	n(%)
CD3	3 (60%)	2 (60%)	5 (100%)	0 (0%)
CD2	4 (80%)	1 (20%)	4 (80%)	1 (20%)
CD4	4 (80%)	1 (20%)	2 (40%)	3 (60%)
CD8	5 (100%)	0 (0%)	4 (80%)	1 (20%)
CD99	4(80%)	1(20%)	3 (60%)	2 (40%)
CD1A	5 (100%)	0 (0%)	4(80%)	1(20%)
CD5	5 (100%)	0 (0%)	4 (80%)	1(20%)
CD34	2 (40%)	3 (60%)	2 (40%)	3 (60%)
CD117	0 (0%)	5(100%)	0 (0%)	5 (100%)
MPO	0 (0%)	5 (100%)	0 (0%)	5 (100%)
CD14	0 (0%)	5 (100%)	0 (0%)	5 (100%)
TDT	2 (40%)	3 (60%)	3 (60%)	2 (40%)
CD22	0 (0%)	5 (100%)	0 (0%)	5 (100%)
CD20	0 (0%)	5 (100%)	0 (0%)	5 (100%)
CD10	2 (40%)	3 (60%)	2 (40%)	3 (60%)
CD33	0 (0%)	5(100%)	0 (0%)	5 (100%)
CD7	5 (100%)	0 (0%)	5 (100%)	0 (0%)
CD79A	0 (0%)	5 (100%)	0 (0%)	5 (100%)
CD19	0 (0%)	5 (100%)	0 (0%)	5 (100%)

T-ALL: T-cell acute lymphoblastic leukemia. IHC: Immunohistochemistry. FC: Flow Cytometry.

CD56 was studied in FC and BM biopsy in 2 patients, and CD123 was studied in FC and BM biopsy in three cases . CD123 was positive in both FC and IHC (**Table 7**). PAX-5 was only studied in BM biopsy in all cases and was found to be negative.

Table 7. Results of antibodies in T-ALL that were studied in both FC and IHC in some patients.

Antibody	Flow-cytometry		Immunohistochemistry	
	+	-	+	-
	n(%)	n(%)	n(%)	n(%)
CD123	0 (0%)	3 (100%)	0 (0%)	3 (100%)
CD56	1(50%)	1 (50%)	0 (0%)	2 (100%)

T-ALL: T-cell acute lymphoblastic leukemia. IHC: Immunohistochemistry. FC: Flow Cytometry.

As a result of both FC and IHC studies in T-ALL in CD117, MPO, CD22, CD14, CD33, CD19, CD20 expressions were found to be negative in all, and CD7 results were positive in all. Among the Kappa Statistic antibodies, CD2, CD10 and CD34 were found to have perfect agreement (k>0.80) (p<0.05) (**Table 8**).

Table 8. Level of agreement of antibodies studied by Flow Cytometry and Immunohistochemical in T-ALL.

Antibody	k	Agreement level	p
CD3	-.154	WEAK	0.361
CD2	1.000	PERFECT	0.025
CD4	0.286	WEAK	0.361
CD99	0.545	LOW LEVEL	0.171
CD10	1.000	PERFECT	0.025
CD34	1.000	PERFECT	0.025
Tdt	0.615	GOOD	0.136

T-ALL: T-cell acute lymphoblastic leukemia. IHC: Immunohistochemistry. FC: Flow Cytometry.

DISCUSSION

In a study conducted with data from 62 countries in 2018, leukemia has been the most common malignancy seen in children aged 0-14 with 284,649 cases out of 140.6 million cases.[2] The immunophenotypic types of childhood acute leukemia have been observed in the literature as 85-86% BCP-ALL.[23,24] In our study, 82.14% of the cases were BCP-ALL. T-ALL rates in the literature ranged between 7.3-27%[25,26] and it was 17.86% in our study. In terms of gender distribution in ALL cases in the literature, it was observed as 55.6% male, 44.4% female and 61.9% male, 38.1% female,[27] and it is more common in male.[28]

In our study, 71.42% of the cases were male and 28.58% were female, and our cases in the male gender were higher compared to the literature. In the study of Noronha et al.[25] the mean age of BCP-ALL cases was four years and the mean age of T-ALL cases was 8 years and in our study, the mean age of BCP-ALL cases (between 2 and 17 years) was 7.22, the age of T-ALL cases was between 3 and 14, with a mean age of 8.80.

The most important method in the classification of acute leukemia is immunophenotyping. Immunophenotyping can be done with both FC in BM aspiration and[21] IHC study in BM biopsy.[6] In our study, the agreement was 100% in terms of biopsy in both BCP-ALL and T-ALL, and leukemia diagnosis in FC. When the literature is examined, studies on this subject are limited, and the diagnostic agreement between the two methods varies between 100%[29] and 95.8%.[30] The most sensitive markers in the diagnosis of BCP-ALL are CD19 and CD79a.[6] In BCP-ALL cases, the presence of 100% and near

100% expression in FC is detected.[21] In our study, CD19 and CD79a in FC were expressed in all our BCP-ALL cases, but CD19 expression was not detected in IHC in only one case.

CD10 is positive in common B-ALL and is approximately 93%.[6,32] In our study, it was found to be 95.7%. In addition, in our study, CD10 was positive in FC in 2 (8.7%) cases, but negative in IHC. In a study in the literature, such a result was found in 1 of 25 ALL cases.[33] CD10 negativity in B-ALL is largely associated with MLL gene rearrangement.

Most BCP-ALL cases have positive expression of CD34 and TdT. In our study, TdT was expressed at a rate of 78.3% and CD34 82.6% in FC in our BCP-ALL cases.[34] In the BM biopsy IHC study, Tdt had positive expression in all BCP-ALL cases. However, in a study in the literature, 18 of the 25 BCP-ALL cases were found to be TdT positive in IHC.[35] TdT expression is absent in approximately 2% of B-ALL cases, and little is known about the clinicopathological and genetic features of this unusual and potentially diagnostically challenging immunophenotypic subtype.

Isolated MPO positivity[36] or false high-density MPO positivity have been reported in BCP-ALL cases while other myeloid markers have been negative.[37]

In all of our cases, MPO was negative in both IHC and FC. Although it is negative in FC, positivity in IHC has been detected in various studies, and these rates go up to 26.7%.[38]

Aberrant expression of CD117 in BCP-ALL is seen as a myeloid marker. Its expression in the literature varies between 0.5-36%.[36,39,40] In this study, it was found to be 13.0% and 17.4% in FC and IHC. CD7 aberrant expression was 2.9% and it was 4.3% in our study.[41] While the expression of CD13 and CD33, myeloid markers, in BCP-ALL was 4.5% and 10.5%, respectively, in our study they were significantly higher than the study and determined to be 26.1% and 30.4%, respectively.[41]

In CD3 and CD14 BCP-ALL, all of our cases were negative in both FC and IHC; they are negative in many studies in the literature, as in our study.[33-35]

CD56 expression in BCP-ALL has generally been associated with poor prognosis. While the[42] expression rate was 11.6% in the study of Aref S et al., it was quite close to another study with 8.7% in both FC and IHC[41] in our study.

CD22 expression in FC and IHC was over 75% in our cases, which was considerably higher than previous studies.[35]

In our study, when the p value was significant (<0.05) in the kappa agreement analysis, good agreement was found in terms of CD20 and CD34 expressions, moderate agreement was found in terms of CD10 expressions, weak agreement was found in terms of CD123 expressions, and very weak agreement was found in terms of CD22 expressions in BCP-ALL.

In this study, myeloid markers MPO, CD14, CD33, CD117, and B lineage markers CD19, CD20, CD22, CD79A were negative in both FC and IHC in T-ALL cases. These results are consistent with previous studies.[7,34,41]

Similar to previous studies, CD5, CD7, CD1a FC were positive in all cases.[41] In IHC, CD3 and CD7 were positive in all cases. In our study, CD10 positivity was 40% in both FC and IHC in T-ALL, and it was found 27.3% in pediatric cases under 14 years old in the previous study, which is lower than our study.[41]

In our study, CD8 was 100% positive and CD4 was 80% positive in FC in T-ALL cases. In the previous study, it was 90% and 72%, respectively.[41] We found CD34 to have 40% positivity in both FC and IHC in T-ALL, which is 9.1%[41] higher compared to the previous study. In our T-ALL cases, we found Tdt positivity as 40% in FC and 60% in IHC, which was relatively low when compared to a study in the literature.[31]

CD99 has been used to detect minimal residual disease (MRD) in various studies. In our T-ALL cases, 80% positivity was found in FC and 60% in IHC. This was determined to be 96% in the previous study, which is higher compared to our study.[43]

In our study, when the p value was significant (< 0.05) in the kappa agreement analysis, perfect agreement was found in terms of CD2, CD10, CD34 expressions, good agreement was found in terms of Tdt expressions, poor agreement was found in terms of CD3, CD4 expressions and low level agreement was found in terms of CD99 expressions in T-ALL.

CONCLUSION

FC is a reliable and rapid method to detect minimal/measurable residual disease (MRD) in diagnosis and post-treatment follow-up in acute leukemia. However, panel selection, high cost and training of the evaluator are the main problems. It is very difficult or impossible to diagnose FC in myelofibrosis, especially when the degree of necrosis is high and bone marrow biopsy cannot be taken. Today, treatments that specifically target surface antigens reduce the expression of these antigens and cause diagnostic difficulties. Therefore, alternative immunophenotyping methods are required.

According to the results of our study, immunophenotyping with IHC in bone marrow biopsy in the new diagnosis of pediatric ALL may be an alternative to immunophenotyping with FC.

In addition, antibodies such as Pax-5, which can only be studied in bone marrow biopsy and are positive in all B-ALL, will be the most important diagnostic tool in the diagnosis of leukemia and bone marrow biopsy in the treatment effects.

Some of the genetic studies on leukemia have been associated with negative cell antigens. For example, in the Tdt negative leukemia group, this negativity was associated with various genes and poor prognosis. In this case, is Tdt negativity immunohistochemical? floow cytometric? should be.

We should look for the answer to this in future studies.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Afyonkarahisar Health Sciences University Clinical Research Ethics Committee (2021/382).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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REFERENCES

1. Nordlund J, Syvänen AC. Epigenetics in pediatric acute lymphoblastic leukemia. Semin Cancer Biol. 2018;51:129-38.
2. Steliarova-Foucher E, Colombet M, Ries LAG, et al. International incidence of childhood cancer, 2001-10: a population-based registry study. Lancet Oncol. 2017;18(6):719-31.
3. Howlader N, Noone A-M, Krapcho M, et al. SEER cancer statistics review, 1975–2010. National Cancer Institute. 2014.
4. Hunger SP, Mullighan CG. Acute Lymphoblastic Leukemia in Children. N Engl J Med. 2015;373(16):1541-52.
5. Iacobucci I, Mullighan CG. Genetic Basis of Acute Lymphoblastic Leukemia. J Clin Oncol. 2017;35(9):975-83.
6. Inaba H, Mullighan CG. Pediatric acute lymphoblastic leukemia. Haematologica. 2020;105(11):2524-39.
7. Jastaniah W, Essa MF, Ballourah W, et al. Incidence trends of childhood acute lymphoblastic leukemia in Saudi Arabia: Increasing incidence or competing risks? Cancer Epidemiol. 2020;67:101764.
8. Loghavi S, Kutok JL, Jorgensen JL. B-acute lymphoblastic leukemia/lymphoblastic lymphoma. Am J Clin Pathol. 2015;144(3):393-410.
9. Rehman A, Abbas N, Saba T, Rahman SIU, Mehmood Z, Kolivand H. Classification of acute lymphoblastic leukemia using deep learning. Microsc Res Tech. 2018;81(11):1310-7.
10. Del Principe MI, De Bellis E, Gurnari C, Buzzati E, Savi A, Consalvo MAI, et al. Applications and efficiency of flow cytometry for leukemia diagnostics. Expert Rev Mol Diagn. 2019;19(12):1089-97.
11. Kremer M, Quintanilla-Martínez L, Nährig J, von Schilling C, Fend F. Immunohistochemistry in bone marrow pathology: a useful adjunct for morphologic diagnosis. Virchows Arch. 2005;447(6):920-37.
12. Cherian S, Soma LA. How I Diagnose Minimal/Measurable Residual Disease in B Lymphoblastic Leukemia/Lymphoma by Flow Cytometry. Am J Clin Pathol. 2021;155(1):38-54.
13. Brestoff JR, Frater JL. Contemporary Challenges in Clinical Flow Cytometry: Small Samples, Big Data, Little Time. J Appl Lab Med. 2022;7(4):931-44.
14. Sitalakshmi S, Srikrishna A, Devi S, Damodar P, Alexander B. The diagnostic utility of bone marrow trephine biopsies. Indian J Pathol Microbiol. 2005;48(2):173-6.
15. Martino M, Alati C, Canale FA, Musuraca G, Martinelli G, Cerchione C. A Review of Clinical Outcomes of CAR T-Cell Therapies for B-Acute Lymphoblastic Leukemia. Int J Mol Sci. 2021;22(4).
16. Tiacci E, Pileri S, Orleth A, et al. PAX5 expression in acute leukemias: higher B-lineage specificity than CD79a and selective association with t(8;21)-acute myelogenous leukemia. Cancer Res. 2004;64(20):7399-404.
17. Gupta GK, Sun X, Yuan CM, Stetler-Stevenson M, Kreitman RJ, Maric I. Usefulness of Dual Immunohistochemistry Staining in Detection of Hairy Cell Leukemia in Bone Marrow. Am J Clin Pathol. 2020;153(3):322-7.
18. Al Gwaiz LA, Bassioni W. Immunophenotyping of acute lymphoblastic leukemia using immunohistochemistry in bone marrow biopsy specimens. Histol Histopathol. 2008;23(10):1223-8.
19. 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(8):455-61.
20. Bain BJ, Barnett D, Linch D, Matutes E, Reilly JT. Revised guideline on immunophenotyping in acute leukaemias and chronic lymphoproliferative disorders. Clin Lab Haematol. 2002;24(1):1-13.
21. Johansson U, Bloxham D, Couzens S, et al. Guidelines on the use of multicolour flow cytometry in the diagnosis of haematological neoplasms. British Committee for Standards in Haematology. Br J Haematol. 2014;165(4):455-88.
22. Alpar C. (2014) Applied statistics and validity and reliability with examples from sports, health and educational sciences. Ankara: Detay Publishing.
23. Wu C, Li W. Genomics and pharmacogenomics of pediatric acute lymphoblastic leukemia. Crit Rev Oncol Hematol. 2018;126:100-11.
24. Schrappe M, Reiter A, Zimmermann M, et al. Long-term results of four consecutive trials in childhood ALL performed by the ALL-BFM study group from 1981 to 1995. Berlin-Frankfurt-Münster. Leukemia. 2000;14(12):2205-22.
25. Noronha EP, Marinho HT, Thomaz EB, Silva CA, Veras GL, Oliveira RA. Immunophenotypic characterization of acute leukemia at a public oncology reference center in Maranhão, northeastern Brazil. Sao Paulo Med J. 2011;129(6):392-401.
26. Tasian SK, Loh ML, Hunger SP. Childhood acute lymphoblastic leukemia: Integrating genomics into therapy. Cancer. 2015;121(20):3577-90.
27. Hsu PC, Pei JS, Chen CC, et al. Association of Matrix Metallopeptidase-2 Promoter Polymorphisms With the Risk of Childhood Leukemia. Anticancer Res. 2019;39(3):1185-90.
28. Köker SA, Oymak Y, Vergi R, Dilek İ, Genel F. The Effects of Immunophenotyping with Flow Cytometry on Prognosis in Acute Lymphoblastic Leukemia. J Contemp Med 11(1):22-8.
29. Mhawech P, Buffone GJ, Khan SP, Gresik MV. Cytochemical staining and flow cytometry methods applied to the diagnosis of acute leukemia in the pediatric population: an assessment of relative usefulness. J Pediatr Hematol Oncol. 2001;23(2):89-92.
30. Kheiri SA, MacKerrell T, Bonagura VR, Fuchs A, Billett HH. Flow cytometry with or without cytochemistry for the diagnosis of acute leukemias? Cytometry. 1998;34(2):82-6.
31. Wimalachandra M, Prabashika M, Dissanayake M, de Silva R, Gooneratne L. Immunophenotypic characterization of acute lymphoblastic leukemia in a flowcytometry reference centre in Sri Lanka. Ceylon Med J. 2020;65(1&2):23-7.
32. Onciu M. Acute lymphoblastic leukemia. Hematol Oncol Clin North Am. 2009;23(4):655-74.
33. Bavikatty NR, Ross CW, Finn WG, Schnitzer B, Singleton TP. Anti-CD10 immunoperoxidase staining of paraffin-embedded acute leukemias: comparison with flow cytometric immunophenotyping. Hum Pathol. 2000;31(9):1051-4.
34. Yasmeen S, Rajkumar A, Grossman H, Szallasi A. Terminal Deoxynucleotidyl Transferase (TdT)-negative Lymphoblastic Leukemia in Pediatric Patients: Incidence and Clinical Significance. Pediatr Dev Pathol. 2017;20(6):463-8.
35. Toth B, Wehrmann M, Kaiserling E, Horny HP. Immunophenotyping of acute lymphoblastic leukaemia in routinely processed bone marrow biopsy specimens. J Clin Pathol. 1999;52(9):688-92.
36. Oberley MJ, Li S, Orgel E, Phei Wee C, Hagiya A, O'Gorman MRG. Clinical Significance of Isolated Myeloperoxidase Expression in Pediatric B-Lymphoblastic Leukemia. Am J Clin Pathol. 2017;147(4):374-81.

37. Savaşan S, Buck S, Gadgeel M, Gabali A. Flow cytometric false myeloperoxidase-positive childhood B-lineage acute lymphoblastic leukemia. Cytometry B Clin Cytom. 2018;94(3):477-83.
38. Fawzy MM, Abd El-hafez A, El-Ashwah S, et al. Isolated Myeloperoxidase Immunohistochemical Expression in Bone Marrow Biopsy Depicts Clinical Outcomes in Adults with Typical B-Acute Lymphoblastic Leukemia. Asian Pac J Cancer Prev. 2021;22(7):2143-52.
39. 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(6):940-9.
40. Basturk A, Akinci S, Hacibekiroglu T, et al. Prognostic significance of flow cytometry findings in Turkish adult acute leukemia patients. Eur Rev Med Pharmacol Sci. 2015;19(18):3360-6.
41. Rezaei MS, Esfandiari N, Refoua S, Shamaei M. Characterization of Immunophenotypic Aberrancies in Adult and Childhood Acute Lymphoblastic Leukemia: Lessons from Regional Variation. Iran J Pathol. 2020;15(1):1-7.
42. Aref S, Azmy E, El-Bakry K, Ibrahim L, Abdel Aziz S. Prognostic impact of CD200 and CD56 expression in pediatric B-cell acute lymphoblastic leukemia patients. Pediatr Hematol Oncol. 2017;34(5):275-85.
43. Roshal M, Fromm JR, Winter S, Dunsmore K, Wood BL. Immaturity associated antigens are lost during induction for T cell lymphoblastic leukemia: implications for minimal residual disease detection. Cytometry Part B: Clinical Cytometry: The Journal of the International Society for Analytical Cytology. 2010;78(3):139-46.