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ORIGINAL ARTICLE

Prognostic Relevance of Immunophenotyping in Adult Patients with Acute Leukemia in Turkey: A Single-Center Experience

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SUMMARY

***Background*: In acute leukemia, many associations have been identified between prognosis and some factors, such as individual antigen expression, cytogenetics, gender, age, high leukocyte level (WBC), platelet count (PLT), and lactate dehydrogenase (LDH), but few are consistent. In this study, we aimed to investigate the cell surface markers and other clinical pathological features for prognosis determination in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) patients in our population.**

***Methods*: This cross-sectional study was performed from January 2017 to December 2023 in İstanbul Training and Research Hospital and included 113 patients (86 AML and 27 ALL) newly diagnosed with AML (non-APL) and ALL. The following tests were fulfilled for the included patients: complete blood count (CBC), LDH, and flow cytometric analysis using a blood sample or bone marrow aspirate. The effects of surface markers, gender, age, WBC, PLT, and LDH on 24-month survival were evaluated retrospectively.**

***Results*: Among the investigated parameters, lack of CD13 expression and positive CD10, cTdT expressions were associated with poor prognosis in AML patients ($p = 0.01$, $p = 0.04$, and $p = 0.04$, respectively). We have found no association between the surface markers and other parameters with prognosis in ALL patients. Age > 65 years was associated with poor prognosis in both AML and ALL patients ($p < 0.001$).**

***Conclusions*: CD10, cTdT positivity, and CD13 negativity may predict poor prognosis for AML and can be considered as prognostic biomarkers for AML patients.**

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INTRODUCTION

Acute leukemia is the cloned expansion of malignant blood cells in the bone marrow, blood, or other tissues. Acute leukemia are divided into types, according to their forms arising from lymphoid or myeloid cell lines. Precursor cells or blast cells from different lineages in acute leukemia express different subsets of surface molecules, many of which are now named as cluster of differentiation (CD) antigens and are identified by flow cytometry [1]. The expression of markers that are not associated with the myeloid or lymphoid lineage, which

are usually found in a part of patients, can be used for both the diagnosis and detection of minimal residual diseases.

Various clinical and biological parameters, including immunophenotype, have been determined over time to predict response to treatment and survival in acute leukemia. These parameters are known to affect prognosis in AML and ALL, such as gender, age, hemoglobin, platelet count, high leukocyte count, lactate dehydrogenase level, cytogenetic abnormality, performance status, and recurrent leukemia [2-3]. However, the prognostic significance of the immunophenotype of blasts in leukemia is still debatable, and a definitive resolution is still being determined. The impact of cell surface markers on disease prognosis is variable, and different findings are obtained in the research carried out. For example, while CD34 positivity in AML is a sign of poor prognosis in some studies [4-6], no positive or negative correlation was found in other studies [7,8]. Aberrant expression of CD19 has been found to be a favorable prognostic marker and has a good treatment outcome in AML [9,10]. However, others have stated that CD19 and CD56 expressions are associated with poor prognosis [10-12]. Although the few early adult ALL studies had demonstrated a poor outcome for My (+) (CD13, CD14, CD15, and CD33) ALL patients [13-15], the other published series demonstrated no prognostic correlation between these two groups [16-18].

In the current study, we aimed to investigate the distribution and abnormal expression of cell surface markers in acute leukemia patients and their relationship with 24-month survival, a prognostic marker. To our knowledge, our study is an uncommon article that investigates the relationship between immunophenotyping and prognosis of adult acute leukemia in Turkey.

MATERIALS AND METHODS

Patients

From January 2017 through December 2023, a total of 113 patients, including 86 untreated patients with de novo AML and 27 with ALL diagnosed in a single institute (İstanbul Training and Research Hospital), were enrolled in this study. Demographic data (age, gender), initial hemoglobin, platelet, leukocyte counts, and LDH levels were recorded. The standard protocol the patients received was "7 + 3″ treatment: 7 + 3 treatment: 1.5 - 3 gr/m^2 Cytarabine, every 12 hours, D1 - 7; 12 mg/m^2 Idarubicin, D1 - 3. In our center, induction protocols that vary depending on age, comorbidities, and performance status have been applied to ALL patients. Acute promyelocytic leukemia (APL) was not included in this study, because those patients received different treatments.

The cases were evaluated as AML and ALL patients in separate groups. In AML cases, the following surface markers CD3, cCD3, CD7, CD10, CD11b, CD13, CD14, CD16, CD19, CD33, CD34, CD56, CD64, CD71, cCD79A CD117, HLADR, MPO, CD45, and cTdT were studied, and for ALL cases the following surface markers CD2, CD3, CD5, CD7, CD9, CD10, CD13, CD15, CD19, CD20, CD21, CD22, CD24, CD33, CD34, CD38, CD56, CD58, CD66c, cCD79A, CD117, CD123, HLA-DR, cMPO, cIgM, cTdT, and CD45 were studied by flow cytometry. The associations between each of these cell surface markers and 24-month survival were evaluated.

Protocols were conducted in accordance with the Declaration of Helsinki. The Ethics Committee of Istanbul Training and Research Hospital approved the study protocol.

Immunophenotyping analysis was performed by using the Navios EX Flow Cytometer (Beckman Coulter) and FACSLyric (Becton, Dickinson Company, CA, USA) model flow cytometry device with 3-lasers (blue, red, and violet), 10 colors. The cells were lysed and stained according to standard protocol. Shortly, the appropriate volume of monoclonal antibody with fluorochrome-conjugate to 100 μL of whole blood or 50 μL was added in a 12 x 75-mm tube, vortexed gently, and incubated in the dark for 15 to 30 minutes at room temperature (20° to 25°C). Then, 2 mL of 1 x FACS Lysing Solution was added, vortexed gently, and incubated for 10 minutes in the dark at room temperature. The solution was centrifuged at 500 x g for 5 minutes. The supernatant was discarded. Two to 3 mL of wash buffer was added and centrifuged at 500 x g for 5 minutes. The supernatant was removed again. Then, 0.5 mL of 1% paraformaldehyde solution was included and mixed carefully, and the solution was stored at 2° to 8°C until acquisition by FACSLyric. For intra-cytoplasmic markers, cells were preprocessed with 500 μL permeabilizing solution and incubated for 10 minutes; after washing, the same procedure was applied for surface markers. Data was obtained and analyzed by FACSuite™ software. The fluorochromes we used were FITC, PC5.5, PE, ECD, PC7, APC, A700, A750, PB, KrO, PERCPCy5.5, APC-H7, V450, and V50, respectively.

Statistical analysis

SPSS software, version 26.0 (IBM Corp., Armonk, NY, USA), was used for all statistical analyses. Clinical and biological factors, including age, gender, leukocyte count, hemoglobin level, platelet count, LDH, and immunophenotypes, were interpreted for their influence on 2-year survival outcomes. Categorical variables were evaluated statistically using the chi-squared test or Fisher's exact tests. A p-value less than 0.05 was regarded statistically significant. Continuous variables with a normal distribution are expressed as the mean ± standard deviation (SD), and non-normally distributed variables are expressed as the median and quartile.

Table 1. Positivity rates of surface markers in AML.

Surface marker	Total patients	Positive patients	Positivity rate
cCD3	79	8	10.1
CD3	38	1	2.6
CD7	41	17	41.5
CD10	81	7	8.6
CD11b	85	40	47
CD13	84	75	89.2
CD14	40	14	35
CD16	41	7	17.1
CD19	80	1	1.2
CD33	81	76	93.8
CD34	81	56	69
CD56	84	25	29.8
CD64	85	51	60
CD71	85	47	55
cCD79a	80	2	2.5
CD117	85	68	80
HLA-DR	85	78	91.7
cMPO	79	63	79.7
CD45	81	81	100
cTdT	81	7	8.6

RESULTS

Among the 86 AML patients, 52 (60.5%) were male and 34 (39,5%) were female, with a mean age of 57.1 ± 14. Additionally, among the 27 ALL patients, 18 (66.7%) were male and 9 (33.3%) were female, with a mean age of 37.3 ± 12.3. Besides the immunophenotype of the study group, we analyzed several clinical and biological characteristics: age, gender, white blood cell count (WBC), hemoglobin, lactate dehydrogenase (LDH), and platelet levels for each patient in this study. WBC counts lower than 30 x 10^9 was for 59 AML and 22 ALL patients and WBC counts higher than 30 x 10^9 was for 25 AML and 5 ALL patients. Forty-two AML patients' LDH enzyme levels were higher than 400 U/L. Sixteen ALL patients' LDH levels were higher than 400 U/L. Platelet level was lower than 50 x 10^9/L in 45 AML patients and in 13 ALL patients.

Table 1 shows the positivity rates of the surface markers in the AML patient group. In AML patients, no statistically significant association was found between prognostic outcome (survival at 24 months) and expression of CD3, cCD3, CD7, CD11b, CD16, CD19, CD33, CD34, CD35, CD36, CD56, CD64, CD71, cCD79a, CD105, CD117, HLA-DR, and IREM2. Beside these, there is a significant negative association between CD10 and AML and cTdT and AML, and a significant positive association between CD13 and AML. ($p = 0.04$, 0.04, 0.01, respectively). Among AML patients, the risk of mortality was found to be significantly increased in patients diagnosed over the age of 65 ($p < 0.001$) (Table 3). Table 2 shows the positivity rates of surface markers in the ALL patient group. No significant relationship was detected between the immunophenotypic markers, biochemical levels, demographic status, and 2-year survival in ALL patients (Table 4).

Table 2. Positivity rates of surface markers in ALL.

Surface marker	Total patients	Positive patients	Positivity rate
CD2	7	3	42.8
CD3	16	3	18.8
CD5	7	4	57
CD7	7	3	42.9
CD9	9	8	88.9
CD10	27	20	74.1
CD13	15	3	20
CD15	9	1	11
CD19	27	20	74
CD20	20	7	35
CD21	9	0	0
CD22	20	19	86.4
CD24	20	13	65
CD33	27	6	22.2
CD34	27	17	65
CD38	9	8	88.9
CD56	7	0	0
CD58	9	7	77.8
CD66c	9	7	77.8
cCD79a	27	20	74
CD117	15	2	13.3
CD123	15	11	73.3
HLA-DR	6	2	33.3
cMPO	27	1	3.7
cIgM	20	4	20
cTdT	27	22	81.5
CD45	27	27	100

DISCUSSION

Although the diagnostic value of immunophenotyping in acute leukemia is certain, the prognostic value of surface antigen expression in acute leukemia is debatable [7-11,19-21]. Several confusing factors can contribute to the discussion, including the number of patient populations studied, methodological differences in antigen detection, single and multicenter data, age, disease complexity, cytogenetics, and treatment protocols. The results of this study demonstrated that immunopheno

Table 3. Antigen expression and demographic biochemical levels in acute myeloid leukemia.

Antigen	No survival	2-year survival	p-value
CD3	1/21	0/17	1
cCD3	4/53	3/31	0.7
CD7	13/24	6/20	0.1
CD10	7/51	0/30	0.04
CD11b	25/52	15/33	0.8
CD13	43/52	32/32	0.01
CD14	6/18	8/22	1
CD16	3/24	4/20	0.68
CD19	1/51	0/29	1
CD33	47/51	29/30	0.6
CD34	36/51	20/30	0.8
CD35	9/22	8/19	1
CD36	28/52	13/30	0.49
CD56	16/51	9/33	0.8
CD64	26/52	22/33	0.18
CD71	29/52	18/33	1
cCD79a	2/51	0/29	0.53
CD105	15/22	7/19	0.06
cMPO	39/50	24/29	0.77
HLA-DR	47/52	31/33	0.7
cTdT	7/51	0/30	0.04
IREM2	5/22	5/19	1
LDH > 400 U/L	27/50	15/32	0.65
LDH < 400 U/L	23/50	17/32	
WBC > 30 x 10⁹/L	17/51	8/33	0.46
WBC < 30 x 10⁹/L	34/51	25/33	
Hb < 8 g/dL	21/51	15/33	0.8
Hb > 8 g/dL	30/51	18/33	
PLT < 50 x 10⁹/L	31/51	14/33	0.12
PLT > 50 x 10⁹/L	20/51	19/33	
Gender – female	19/52	14/33	0.6
Gender – male	33/52	19/33	
Age > 65	21/52	1/33	< 0.001
Age < 65	31/52	22/33	

typing may be useful in determining prognosis in acute leukemia. The results showed that in lymphoid or aberrant marker positive (CD7, CD10, CD19, and CD56) AML, CD7 was the most common (41.7%) as compared to other lymphoid markers. However, in positive myeloid or aberrant markers (CD13, CD14, CD15, and CD33) ALL, CD33 was the most common (22%) compared to other myeloid markers. In our study on AML patients, among the surface antigens we studied, CD13 expression was associated with good prognostic outcomes, confirming previous studies [13,22] and CD10 (an aberrant marker for AML) and cTDT expression were found to be poor prognostic markers. CD13 is a membrane-bound zinc-binding metalloprotease expressed on early processed granulocytes-monocyte progenitors and maturing cells of these lineages, which cleaves regulatory peptides expressed in the blast cells of most cases of AML (95%) [23]. The expression rate in our AML study population was 89.2%. Confirming our study, Schwarzinger et al. demonstrated that the percentage of CD13 expression has been identified as an important indicator for achieving complete remission (CR)

Table 4. Antigen expression and demographic biochemical levels in acute lymphoblastic leukemia.

Marker	No survival	2-year survival	p-value
CD7	3/6	5/10	1
CD10	9/12	11/15	1
CD33	4/12	2/15	0.36
CD34	7/12	10/15	0.7
cCD79a	10/12	10/15	0.4
cTdT	11/12	11/15	0.34
cMPO	1/12	0/15	0.44
CD117	1/6	1/9	1
CD123	5/6	6/9	0.6
Gender - male	9/12	9/15	0.68
Gender - female	3/12	6/15	
Age > 65	0/12	3/15	0.23
Age < 65	12/12	13/15	
Hg < 8 g/dL	6/12	5/15	0.45
Hg > 8 g/dL	6/12	10/15	
LDH < 400 U/L	3/11	7/15	0.43
LDH > 400 U/L	8/11	8/15	
PLT < 50 x 10^9/L	7/12	6/15	0.45
PLT > 50 x 10^9/L	5/12	9/15	

[13]. In Marosi et al.'s study, all but one achieved complete remission, significantly more than patients lacking this phenotype ($p < 0.001$) [22]. In Jiang et al.'s study, the lack of CD13 was an independent predictor for shorter overall survival (OS) [25]. According to Cui et al.'s study, the frequently altered leukemia-associated aberrant immunophenotype (LAIPs) was a lack of lineage-specific antigen and lineage infidelity LAIP changes are common in refractory and relapsed AML. The most frequently changed marker was CD13, followed by CD33, CD56, CD7, CD4, and CD11b [27]. Another study by Legrand et al. found no correlation between CD13 and clinical outcome or other prognostic factors [26].

Terminal deoxynucleotidyl transferase (TdT) interacts with the nuclei of regular T and B lymphocyte precursors and their neoplastic counterparts. About 18% of AML patients have a positive TdT marker [26]. In our AML population, the cytoplasmic form of this antigen's expression was 8.7%. In Venditti et al.'s study, the expression of TdT is associated with an unfavorable outcome, like our study, and co-expression of TdT with CD7 indicated a subclass of AML with a poor prognosis. However, they did not find any association between prognosis and CD10 expression [19]. In Legrand et al.'s and Chang et al.'s studies, there is no association between prognosis and TdT expression [26,28]. The clinical impact of TdT expression on AML outcomes remains unclear. Saburi et al.'s study suggests that expression of TdT is related to an increased risk of relapse in intermediate-risk AML patients [37].

CD10 is a 100 kDa glycoprotein and is also known as common acute lymphocytic leukemia antigen (CALLA). This is a cell surface enzyme with neutral metalloendopeptidase activity that suppresses various biologically active peptides [29]. It is an aberrant marker for AML, and in our study, its expression (8.6%) is associated with poor diagnosis in the AML patient group. Webber et al. found that CD10 is not a predictor antigen for relapse in the AML group [30]. According to Repp et al.'s study, CD10 significantly negatively impacts complete remission rates in AML, confirming our study [30].

According to Fang et al.'s study, CD56(+), and according to Xu et al.'s study, CD56(+), CD34(+) AML patients respond poorly to treatment, frequently relapse after complete remission (CR), have a low survival rate, and CD19 (+) AML patients have a good treatment outcome [10,32]. In Wang et al.'s and Rai et al.'s studies, besides CD34 and CD56, there is also a negative association between CR rates of patients positive for CD7 and CD25 and a positive association between CR rates and MPO and CD19 [33,34]. In Sun et al.'s study, high expression of CD56 is related with unfavorable clinical outcomes in de novo non-M3 AML patients [35]. In Dang et al.'s study [36], CD34 expression has a nega-

tive impact on prognosis in nucleophosmin 1 (NPM1) mutation-positive AML group. However, in another study [12], CD7 was not associated with a good or bad prognosis in 7 patients with positive CD7 expression, out of a total of 40 AML patients. We found no correlation between these markers (CD56, CD34, CD19, CD7, and MPO) and the prognosis marker in our AML patient group. A low number of patient groups, cytogenetic differences, and using only a 2-year survival rate for the prognostic marker may play a role in this outcome. Our findings do not support previous reports attributing poor prognosis to AML cases with myeloblasts expressing CD34 and HLA-DR, hematopoietic progenitor cell markers [22,24-26].

Xu et al. showed in their meta-analysis that the degree of CD11b expression is closely associated to the prognosis of AML patients and can be used as a prognostic biomarker to classify AML patients [38]. In our study, for the 2-year survival status, which we used as a prognostic marker, no significant difference was found in terms of CD11b expression in the AML patient groups ($p = 0.8$).

While ALL ranks first among childhood leukemia, it constitutes 20% of adult leukemia. Basically, it is classified as a T and B cell based on the lymphoid cell from which it originates. While its incidence is 1 - 5/100,000 worldwide, more than 2/3 of the cases are of B cell origin. In ALL patients, we could not demonstrate any association between any surface antigen expression and prognosis. This may be related to the small number of patient groups. Tanyeli et al.'s study could not find any association between the positivity of myeloid antigens and clinical and laboratory characteristics of ALL [39]. In Khaled et al.'s study, the B-ALL patient group with CD13 and CD33 expressions had significantly higher complete remission rates [21].

Our results confirmed that older age is an adverse prognostic parameter in AML. The prognostic relevance of immunophenotypes to FAB subtypes of leukemia were not investigated in our group.

CONCLUSION

Our findings show that in AML patients, the expression of CD10 and TdT markers and the lack of CD13 expression may be poor prognosis indicators, when the markers are evaluated alone. Further studies involving a higher number of patient groups, examining the correlation with genetics, and also including co-expressions are required. This study may be one of the rare and pioneering studies examining prognostic factors for acute leukemia patients in the Turkish adult population.

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Declaration of Interest:
The authors have declared that no competing interests exist.

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