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Prognostic significance of flow cytometry findings in Turkish adult acute leukemia patients

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Abstract. – OBJECTIVE: Several factors are known to affect prognosis of acute leukemia such as age, high leukocyte count, cytogenetic abnormality, performance status and recurrent leukemia.

We aimed to investigate the association between cell surface markers and prognostic determinants such as recurrence at 6 and 12 months and survival at 6, 12 and 18 months in acute leukemia patients.

PATIENTS AND METHODS: A total of 142 patients, 101 with acute myeloid leukemia (AML) and 41 with B-cell acute lymphoblastic leukemia (B-ALL) were included. The effects of surface markers on survival and recurrence rates were evaluated retrospectively.

RESULTS: In AML patients, CD5+ and CD34+ immunophenotypes and in ALL patients cCD22+, CD34+ and CD49f + CD19+ immunophenotypes were positive prognostic indicators. In AML patients CD7 expression, and in ALL patients CD5+, CD7+ and CD117+ immunophenotypes and >90% CD45 expression were negative prognostic indicators.

CONCLUSIONS: This study demonstrates that flow cytometry, a common diagnostic tool in acute leukemia, may also have prognostic value in acute leukemia in the future.

Key Words:
Acute leukemia, Flow cytometry, Immunophenotype, Turkish.

Introduction

Several factors are known to affect prognosis in acute leukemia such as age, high leukocyte count, cytogenetic abnormality, performance status and recurrent leukemia[1-5]. Immunophenotypic characteristics also influence prognosis. The effect of cell surface markers on prognosis is variable and dependent on other factors. For example, expression of CD34 is an accepted marker of poor prognosis in acute myeloid leukemia (AML)[6] despite some reports demonstrated no association between CD34 expression and AML prognosis[7,8]. Aberrant expression of CD19 have been found to be positive prognostic indicators in AML[9] but others have reported an association between CD19 expression and poor prognosis[10]. Mature B-cell subtype immunophenotype has strong prognostic and therapeutic significance in B-cell acute lymphoblastic leukemia (B-ALL)[11]. Other immunophenotypic markers have variable rates of prognostic significance. CD34 expression is most commonly observed in adult B-ALL patients and the Cancer and Leukemia Group B (CALGB) study group reported an association between CD34 expression and poor prognosis[12].

In this study, the association between cell surface markers and prognostic determinants such as recurrence at 6 and 12 months and survival at 6, 12 and 18 months were evaluated in acute leukemia patients. To our knowledge, this is the first article about immunophenotyping and prognosis of adult acute leukemias in Turkey.

Patients and Methods

A total of 142 patients, 101 with AML and 41 with B-ALL, that treated with standard

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chemotherapy protocols between 2009 and 2013 at Ankara Atatürk Training and Research Hospital, Ankara Numune Training and Research Hospital and Dı kapı Yıldırım Beyazıt Training and Research Hospital were included and retrospectively evaluated. Standard AML induction therapy comprised idarubicine 12 mg/m^2 for 3 days and cytarabine 100 mg/m^2 for 7 days. Consolidation therapy comprised 2-4 cure high-dose cytarabine 3 g/m^2 on days 1, 3 and 5. Standard B-ALL therapy was administered as described in the International MRC UKALL XII/ECOG E2993 trial or CALGB.

Patients with acute promyelocytic leukemia and those who could not receive standard treatment due to older age or poor performance status were excluded.

Cases were evaluated in two groups as AML and ALL. In AML cases the following surface markers CD2, CD3, CD5, CD7, CD10, CD11b, CD13, CD14, CD15, CD19, CD20, CD24, CD33, CD34, CD56, CD64, CD117, HLADR, MPO and CD45 and for ALL cases the following surface markers CD2, CD3, CD5, CD7, CD10, CD13, CD15, CD19, CD10+CD19, CD20, CD22, CCD22, CD24, CD33, CD34, CD38, CD49F+CD19, CD56, CD79A, CCD79A, CD117, SIGM, CMU, HLA-DR, MPO, TdT and CD45 were studied by flow cytometry. The association between these cell surface markers and recurrence at 6 and 12 months and survival at 6, 12 and 18 months was evaluated.

Statistical Analysis

Immunophenotyping analysis was performed by using a 2-laser, 6-colour, Beckman Coulter, Navios model flow cytometry device. SPSS 17 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Variance was calculated as the mean ± standard deviation (SD). Percentages were reported in addition to numerical data in frequency analyses. Categorical data were analysed using the Chi-squared test. $p < 0.05$ was considered statistically significant.

Results

The out of 101 AML patients, 59 (58.4%) were male and 42 (41.6%) were female with a mean age of 48.5 ± 14. The out of 41 ALL patients, 28 (68.3%) were male and 13 (31.7%) were female with a mean age of 37.7 ± 15.

Expression of the surface markers CD10, CD20 and CD3 (n = 97, n = 43; n = 36, respectively) was not observed in any AML patients. Positivity rates of surface markers in AML are given in Table I.

In AML patients no statistically significant association was found between prognostic outcomes (recurrence at 6 and 12 months and survival at 6, 12 and 18 months) and expression of CD2, CD3, CD11b, CD13, CD14, CD19, CD24, CD56, CD64, CD117, HLA-DR or MPO.

Findings of AML patients: The 6-month recurrence rate in CD34$^+$ patients was significantly lower than that in CD34$^-$ patients (6.9% *vs.* 30.8%, $p = 0.041$).

The 6-month survival rate in CD5$^+$ patients was significantly higher than that in CD5$^-$ patients (83.3% *vs.* 36%, $p = 0.03$). The 18-month survival rate of CD7$^+$ patients trended towards being lower than that of CD7$^-$ patients (5% *vs.* 19.7%, $p = 0.062$). Cell surface markers identified as good and poor predictors of AML prognosis are given in Table II.

Findings of B-ALL patients: Expression of CD3 and MPO (n = 11 and 39, respectively) was not observed in any cases of B-ALL. Positivity rates of surface markers in B-ALL are given in Table III.

In B-ALL patients no statistically significant association was found between prognostic outcomes (recurrence at 6 and 12 months and survival at 6, 12 and 18 months) and expression of

Table I. Positivity rates of surface markers in AML

	Total patients	Positive patients	Positivity rate
CD2	95	12	12.60%
CD3	36	0	0%
CD5	44	6	13.60%
CD7	96	35	36.50%
CD10	97	0	0%
CD11b	93	50	53.80%
CD13	97	87	89.70%
CD14	95	22	23.20%
CD15	94	58	61.70%
CD19	97	10	10.30%
CD20	43	0	0%
CD24	41	7	17.10%
CD33	96	92	95.80%
CD34	97	65	67%
CD56	94	20	21.30%
CD64	95	56	58.90%
CD117	96	86	89.60%
HLA-DR	97	92	94.80%
MPO	96	71	74%
CD45	97	96	99%

Table II. Cell surface markers identified as good and poor predictors of AML and B-ALL prognosis.

For AML patients				
Cell surface marker	**Good prognosis**	**Poor prognosis**	**Evaluation criteria**	***p* value**
CD34	+		6-month relapse	$p = 0.041$
CD5	+		6-month survival	$p = 0.03$
CD7		+	18-month survival	$p = 0.062$
For B-ALL patients				
Cell surface marker	**Good prognosis**	**Poor prognosis**	**Evaluation criteria**	***p* value**
CD7		+	6-month relapse	$p = 0.012$
CD117		+	6-month relapse	$p = 0.005$
CD34	+		6-month relapse	$p = 0.011$
CD7		+	6-month survival	$p = 0.049$
cCD22	+		6-month survival	$p = 0.044$
CD34	+		6-month survival	$p = 0.040$
CD49f+CD19	+		6-month survival	$p = 0.020$
CD5		+	12-month survival	$p = 0.019$
CD7		+	12-month survival	$p = 0.034$
CD34	+		12-month survival	$p = 0.017$
CD117		+	12-month survival	$p = 0.010$
CD45>%90		+	12-month survival	$p = 0.044$
CD45>%90		+	18-month survival	$p = 0.003$

CD10, CD15, CD19, CD10+CD19, CD20, CD22, CD24, CD49f, CD79a, HLA-DR, MPO or TDT. Cell surface markers identified as good and poor predictors of B-ALL prognosis are given in Table II.

The 12-month survival rate in $CD5^+$ patients was significantly lower than that in $CD5^-$ patients (0% *vs.* 43.7%, $p = 0.019$). The 6-month relapse rate in $CD7^+$ patients was significantly higher than that in $CD7^-$ patients (100% *vs.* 15.4%, $p = 0.012$). The 6- and 12-month survival rates in $CD7^+$ patients were significantly lower than those in $CD7^-$ patients (16.7% *vs.* 60%, $p = 0.049$ and 0% *vs.* 45.7%, $p = 0.034$, respectively). The 6-month survival rate in $cCD22^+$ patients was significantly higher than that in $cCD22^-$ patients (80% *vs.* 43.3%, $p = 0.044$).

The 6-month recurrence rate in $CD34^+$ patients was significantly lower than that in $CD34^-$ patients (9.1% *vs.* 75%, $p = 0.011$). The 6 and 12-month survival rates in $CD34^+$ patients were significantly higher than those in $CD34^-$ patients (63.3% *vs.* 27.3%, $p = 0.04$ and 50% *vs.* 9.1%, $p = 0.017$, respectively).

The 6-month survival rate in CD49f + $CD19^+$ patients was significantly higher than that in CD49f + $CD19^-$ patients (75% *vs.* 20%, $p = 0.02$).

The 6-month recurrence rate in $CD117^+$ patients was significantly higher than that in $CD117^-$ patients (100% *vs.* 0%, $p = 0.005$). The

Table III. Positivity rates of surface markers in B-ALL.

	Total patients	**Positive patients**	**Positivity rate**
CD2	27	1	3.70%
CD3	11	0	0%
CD5	25	9	36%
CD7	41	6	14.60%
CD10	41	38	92.70%
CD13	41	6	14.60%
CD15	26	2	7.70%
CD19	41	41	100%
CD10+CD19	40	37	92.50%
CD20	37	30	81.10%
CD22	41	40	97.60%
cCD22	40	10	25%
CD24	15	14	93.30%
CD33	40	8	20%
CD34	41	30	73.20%
CD49f	11	1	9.10%
CD49f+CD19	18	8	44.40%
CD38	18	17	94.40%
CD56	22	1	4.50%
CD79a	41	3	7.30%
cCD79a	40	39	97.50%
CD117	25	9	36%
HLA-DR	39	38	97.40%
MPO	39	0	0%
s gM	38	11	28.90%
cmü	38	32	84.20%
TDT	41	28	68.30%
CD45	41	36	87%
CD45%90	41	28	68.20%

12-month survival rate in $CD117^+$ patients was significantly lower than that in $CD117^-$ patients (0% *vs.* 50%, $p = 0.010$).

The threshold for CD45 positivity was defined as >90%. The 12 and 18-month survival rates in patients with >90% CD45 was significantly lower than those in patients with <90% CD45 (28.6% *vs.* 61.5%, $p = 0.044$ and 3.6% *vs.* 38.5%, $p = 0.003$).

In B-ALL patients, interestingly all $CD5^+$, $CD7^+$, and $CD117^+$, identified as poor prognostic predictors, was observed to accumulate in 13 cases. However, there was no positive or negative correlation between these markers and other parameters.

Discussion

The results of this study demonstrate immunophenotyping has utility in determining prognosis in both AML and ALL. In AML patients, CD5 and CD34 expression was associated with good prognostic outcomes and CD7 expression was found to be a poor prognostic indicator. In ALL patients, $cCD22^+$, $CD34^+$ and CD49f + $CD19^+$ was associated with a good prognostic outcomes, and $CD5^+$, $CD7^+$ and $CD117^+$ and >90% CD45 expression was associated with poor prognostic outcomes.

CD5 expression is not common in AML. CD5 was found to be positively expressed in 6 of the 832 AML patients in a previous study but the prognostic significance of CD5 expression was not reported[13]. In another work[14], CD5 expression was observed in 5 out of 61 patients and CD5 expression was suggested as a negative prognostic indicator of survival. In our study, aberrant expression of CD5 was observed in 6 out of 44 AML patients (13.6%). In AML patients, the overall 6-month survival rate in $CD5^+$ patients was statistically significantly higher than that in $CD5^-$ patients ($p = 0.03$). Therefore, we propose CD5 expression as a positive prognostic indicator in AML patients.

CD7, generally expressed on mature T and natural killer lymphocytes, is found in 15%-20% of AML cases. CD7 expression has been shown to be associated with poor prognosis in AML particularly in undifferentiated subtypes (M0, M1) with CD34, MDR-1 or TdT expression[15,16]. Further, CD7 expression has been shown to be closely associated with FLT3 mutations[17,18]. In another study[19] involving 154 AML patients, CD7 expression was found to be associated with shortened survival. However in another study (20), CD7 expression was found in 7 of 40 AML patients and was not associated with good or poor prognosis. In our study, CD7 expression was observed in 35 of 96 (36%) AML patients and although not statistically significant, the 18-month survival rate was lower in $CD7^+$ patients than that in $CD7^-$ patients ($p = 0.062$).

CD34 expression has been reported as a poor prognostic indicator in AML, although there are conflicting data (21,22). In our study, CD34 expression was observed in 65 out of 97 (67%) AML patients. The 6-month recurrence rate was lower in $CD34^+$ patients than that in $CD34^-$ patients ($p = 0.041$).

In ALL cases, detailed evaluation of the association between prognosis and immunophenotypic features is not present. The small number of reports that have evaluated the association between prognosis and CD5 expression in ALL cases have suggested CD5 expression as a poor prognostic indicator (23-25). In our study, 9 out of 25 ALL patients (36%) had $CD5^+$. The 12-month survival rate in $CD5^+$ patients was significantly lower than those with $CD5^-$ patients ($p = 0.019$).

Few studies have examined the association between prognosis and CD7 expression in ALL. One study (25) reported that CD7 is rarely expressed in B-ALL patients, observed in 6 out of 134 B-ALL patients, and was proposed as marker of poor prognosis. Seegmiller et al[24] reported CD7 expression in 4 out of 200 B-ALL cases but they didn't reported any prognostic value of CD7. In our study, 6 out of 41 B-ALL patients had $CD7^+$. The 6-month recurrence rate was higher and the 6- and 12-month survival rates were lower in $CD7^+$ patients than those in $CD7^-$ patients ($p = 0.012$, $p = 0.049$ and $p = 0.034$, respectively).

The function of CD22 is not fully understood but is thought to be a component of the B-cell activation complex and may be an adhesion molecule. Decreased numbers of mature B cells and increased apoptosis have been shown in the peripheral circulation and bone marrow of mice lacking CD22[26,27]. In one research[28], the presence of Philadelphia chromosome was found to be correlated with CD22 positivity in ALL patients. In a paper[29] evaluating 795 children with B-ALL, the expression of CD34 was associated with good prognosis and CD22 expression was found to be correlated with CD34 expression. We are

not aware of any study examining the impact of cCD22 on prognosis except in these reports. In our study, cCD22$^+$ was observed in 10 out of 40 (25%) B-ALL patients. Expression of cCD22 was associated with a higher 6-month survival rate in B-ALL patients ($p = 0.044$).

There are conflicting opinions regarding the relationship between CD34 expression and prognosis in ALL[30-33]. Recent studies suggest that CD34 has no prognostic value in B-ALL cases (34,35). In our study, 30 out of 41 B-ALL patients (73.2%) had CD34$^+$. The 6-month recurrence rate was lower, and the 6- and 12-month survival rates were higher, in CD34$^+$ patients than those in CD34$^-$ negative patients. CD34 expression was associated with more favourable prognosis by 6-month recurrence rates and 6 and 12-month survival rates ($p = 0.04$ and $p = 0.017$, respectively).

CD45 is a transmembrane cell surface protein with tyrosine phosphatase activity. In a study evaluated 529 B-ALL patients, CD45 expression was not found to be associated with prognosis[36]. But in another work[37] evaluating children with ALL, patients with > 90% (considered positive) CD45 expression had a poorer prognosis than those patients with < 90% CD45 expression. Recently similar results were reported by other authors[38]. In our study, there was no significant association between prognosis and CD45 expression. However, the 12- and 18-month survival rates in patients with > 90% CD45 expression were lower than those in patients with < 90% CD45 expression ($p = 0.044$ and $p = 0.003$, respectively).

CD49f + CD19$^+$ was observed in 8 out of 16 B-ALL patients. These positive cases had higher 6-month survival rates than those CD49f + CD19$^-$ cases ($p = 0.02$).

CD117 is expressed by myeloid progenitor cells and is considered specific for myeloid lineages. CD117 can be aberrantly expressed in B-ALL[39]. In a previous study[24], CD117 expression was detected in only 1 out of 200 B-ALL patients. In another report[40], CD117 expression was observed in 4 out of 183 B-ALL patients, however its impact on prognosis was not reported. CD117 expression, rarely seen in B-ALL, was found in 9 out of 25 (36%) B-ALL patients in our study. The 6-month recurrence rate was higher and 12-month survival rate was lower in CD117$^+$ B-ALL patients than those in CD117$^-$ patients ($p = 0.005$ and $p = 0.010$, respectively).

In literature some surface markers are correlated with certain leukemia subtypes[41]. In our study, positive prognostic value of these surface markers may be associated with certain FAB subtypes or with certain cytogenetic abnormalities. Unfortunately we could not obtain the FAB subtypes and cytogenetic abnormalities of our patients.

Further researches utilizing surface markers and cytogenetics of leukemic cells are needed to better define the prognostic factors of patients with leukemia.

Conclusions

These results demonstrate CD5 and CD34 expressions are positive prognostic indicators in AML. CD5 is a poor and CD34 is a good prognostic indicator in B-ALL patients. Aberrant expression of CD7 is a poor prognostic indicator in both leukemias. In B-ALL patients, cCD22 and CD49f + CD19 are good, and CD117 and > 90% CD45 expressions are poor prognostic indicators. In conclusion, we propose that flow cytometry, a commonly used diagnostic tool for acute leukemia, may also have prognostic value in acute leukemia in the future.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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