Original Article

DOI: 10.14235/bas.galenos.2021.5875
Bezmialem Science 2022;10(4):402-8

Prognostic Significance of Flow Cytometric Immunophenotyping in Patients with Acute Myeloid Leukemia

Akut Miyeloid Lösemili Hastalarda Akış Sitometrik İmmünofenotiplemenin Prognostik Önemi

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ABSTRACT

Objective: Chromosomal abnormalities are one of the most important prognostic factors in acute myeloid leukemia (AML). However, not all patients may have such informative chromosomal abnormalities. Although there are many studies on the prognostic value of immunophenotyping in AML, it is still not used as a prognostic marker. In this study, we aimed to investigate the effects of CD13, CD33, CD34, CD117, MPO and HLADR expressions on prognosis of non-acute promyelocytic leukemia AML.

Methods: One hundred thirteen patients diagnosed as having non-acute promyelocytic leukemia AML and followed up between 2010 and 2018 were included in this study. The associations of CD13, CD33, CD34, CD117, MPO and HLA DR expressions with chemotherapy response, progression free survival (PFS) and overall survival (OS) were statistically analyzed.

Results: It was seen that response to chemotherapy was achieved in 67.3% of the patients. Median PFS duration was 9 months and median OS duration was found as 13 months. Of the immunophenotypic characteristics, only MPO expression was determined to be an independent risk factor for PFS and OS.

Conclusion: Immunophenotypic features may be helpful in the diagnosis of AML as well as give an idea about prognosis. In this

ÖZ

Amaç: Kromozomal anormallikler, akut miyeloid lösemide (AML) en önemli prognostik faktörlerden biridir. Bununla birlikte, tüm hastalarda bu tür bilgilendirici kromozomal anormallikler olmayabilir. AML'de immünofenotiplemenin prognostik değeri üzerine birçok çalışma olmasına rağmen, immünofenotipleme halen prognostik belirteç olarak kullanılmamaktadır. Bu çalışmada akut promiyelositik lösemi dışı AML'de CD13, CD33, CD34, CD117, MPO ve HLADR ekspresyonlarının prognoz üzerindeki etkilerini araştırmayı amaçladık.

Yöntemler: Bu çalışmaya 2010-2018 yılları arasında tanı konup tedavi edilen 113 akut promiyelositik lösemi dışı AML'li hasta dahil edilmiştir. CD13, CD33, CD34, CD117, MPO ve HLA DR ekspresyonlarının kemoterapi yanıtı ve progresyonsuz (PFS) ve genel sağkalım (OS) ile ilişkisi istatistiksel olarak analiz edildi.

Bulgular: Hastaların %67,3'ünde kemoterapiye yanıt alındığı görüldü. Medyan PFS süresi 9 aydı ve medyan OS süresi 13 ay olarak bulundu. İmmünofenotipik özelliklerden yalnızca MPO ekspresyonunun, PFS ve OS için bağımsız bir risk faktörü olduğu belirlendi.

Sonuç: İmmünofenotipik özellikler AML tanısında yardımcı olabileceği gibi prognoz hakkında da fikir verebilir. Bu çalışmada,

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Received: 07.01.2021
Accepted: 02.08.2021

Cite this article as: Demircioğlu S, Ekinci Ö, Doğan A, Ulaş T. Prognostic Significance of Flow Cytometric Immunophenotyping in Patients with Acute Myeloid Leukemia. Bezmialem Science 2022;10(4):402-8

study, MPO expression was shown to be an independent risk factor for PFS and OS in our own patient population.

Keywords: Acute myeloid leukemia, immunophenotyping, prognosis, survival

MPO ekspresyonunun kendi hasta popülasyonumuzda PFS ve OS için bağımsız bir risk faktörü olduğu gösterilmiştir.

Anahtar Sözcükler: Akut miyeloid lösemi, immünofenotipleme, prognoz, sağkalım

Introduction

Acute myeloid leukemia (AML) is a malignant disorder of hematopoietic stem cells characterized by clonal proliferation of abnormally differentiated myeloid series blasts. AML is one of the most common types of leukemia in adults. There are approximately 19,940 new cases of AML and approximately 11,180 deaths from AML annually in the United States (1). Treatment response and overall survival (OS) in AML are heterogeneous. A number of prognostic factors have been identified for patient and tumor characteristics for AML, including age, performance status, and karyotype (2-4). Advanced age (>60), poor performance status, treatment-related AML, myelodysplasia, or AML after myeloproliferative neoplasms are known to be poor prognostic factors (5-7). Today, AML risk classification is performed based on cytogenetic and molecular properties. Criteria published by European LeukemiaNet (ELN) group in 2017 is used for risk classification (8).

In recent years, immunophenotyping has become a standard practice in the diagnosis of hematologic neoplasms and in the definition of the origin of cells. The clinical significance of surface antigen expression has been investigated for more than 20 years, but so far several consistent results have been achieved (9). The markers with significant prognostic associations demonstrated in multiple studies were CD13, CD14, and CD15 (10-13). Many other markers claimed to have prognostic significance, alone or in combination, have not been supported in other studies. In this study, we aimed to investigate the effects of CD13, CD33, CD34, CD117, MPO and HLADR expressions on prognosis of non-acute promyelocytic leukemia AML.

Methods

Patients

One hundred thirteen patients diagnosed as having non acute promyelocytic leukemia AML and followed up between 2010 and 2018 were included in this study. The gender, age at diagnosis, complete blood count at the time of diagnosis, flow cytometry results, risk groups, treatment responses, PFS, OS, and final status were obtained retrospectively from the patient files. Approval was received from the Non-invasive Clinical Research Ethics Board at Van Yüzüncü Yıl with the date 07.12.2018 and approval number 10.

Risk groups were determined according to ELN 2017. t(8; 21), t(15; 17), inv16, NPM1 mutation without FLT3-ITD mutation, and those with biallelic CEBPA mutation were considered as good risk groups. Co-mutation in NPM1 and FLT3-ITD, t(9; 11) and presence of cytogenetic abnormalities not classified as positive or negative were accepted as standard risk groups. t(6; 9), t(v; 11q23.3), t(9; 22), inv(3), -5 or del(5q), -7; -17/abn(17p), complex karyotype, monosomal karyotype, presence of FLT3-ITD mutation without NPM1 mutation, and presence of RUNX1, ASXL1, TP53 mutations were considered as bad risk group (8). ELN 2017 risk classification was evaluated with conventional cytogenetics, FISH and next-generation sequencer. RUNX1, ASXL1, TP53 could not be studied due to technical impossibilities.

OS was calculated from diagnosis to death. Progression free survival (PFS) was defined as the time from start of treatment to disease progression or death from any cause. Survivals were expressed in months.

As induction chemotherapy, 73% of the patients received idarabucine (2 or 3 days) + cytarabine (5 or 7 days) chemotherapy, 27% received azacitidine (7 days) or desitabine (5 days). Those who did not remitter after idarabucine + cytrabine CT were given reinduction chemotherapy with the same chemotherapy regimen. After remission was achieved, patients in the high-risk group had allogeneic stem cell transplantation if donor was present. Patients without high risk were consalidated with 3-4 cycles of high-dose cytarabine. Patients receiving azacitidine or decitabine were evaluated for response after 4-6 cycles. Treatment was continued with responders until progression.

Immunophenotypic Analysis

Flow cytometric immunophenotyping was performed on bone marrow aspirate. Specimen processing was performed according to a routine red cell lysis protocol. Single cell suspensions were stained with various 4 fluorochrome-conjugated antibody combinations and analyzed in reference to isotype-matched fluorochrome-conjugated control antibodies. Immunophenotypic properties were evaluated with Multiparametric Flow Cytometry (Facs Canto II, BD, Brussels, Belgium). CD3, CD5, CD7, CD19, CD22, CD13, CD33, CD14, CD15, CD16, CD34, CD41, CD56, CD64, CD117, MPO, Tdt, and HLADR expressions were studied in the acute leukemia panel. While expressions >20% were considered positive, <20% was considered negative.

Statistical Analysis

While our study expressed the continuous variables with their descriptive characteristics as median, minimum and maximum values, the categorical variables were expressed in terms of frequencies and percentages. The chi-square test was performed to detect the relationship between response rates to the treatments and immunophenotypic features; and also detect the relationship between the risk groups and the survival rates. The PFS and OS curves were constructed by Kaplan-Meier method and differences among groups were calculated by using log-rank

test. The Cox proportional hazard regression model was used to examine the potential prognostic factors for outcome. A two-sided p value of <0.05 was accepted to be statistically significant. All analyses were performed using SPSS software (version 24.0; IBM Corp., Armonk, NY, USA).

Results

Fifty four (47.8%) of the patients were female and 59 (52.2%) were male. The median age at diagnosis was 51 (18-81) years. Other descriptive properties are given in Table 1.

The mean PFS duration was 9 (0-113) months and the mean OS duration was found as 13 (1-115) months. There was no statistical difference between response to chemotherapy and the expression of CD13 (p=0.356), CD33 (p=0.676), CD34 (p=0.256), CD117 (p=0.108), MPO (p=0.246), and HLADR (p=0.154).

The OS and PFS differences were observed between the risk groups (Table 2). PFS was longer in favorable risk group than intermediate and adverse risk groups (p=0.036 and p=0.007, respectively). No difference was observed in terms of PFS between the intermediate risk group and adverse risk group (p=0.16). In favorable risk group, OS was longer than intermediate and adverse risk groups (p=0.001 and p=0.00, respectively). There was no difference in terms of OS between the intermediate risk group and the adverse risk group (p=0.09) (Table 2).

The MPO expression was found to be an independent risk factor on PFS and OS (p=0.008 and p=0.015, respectively) (Table 3). In the survival analysis, PFS and OS were longer in the group expressing MPO (p=0.009 and p=0.004, respectively) (Figure 1). Although patients with HLADR negativity had higher PFS and OS advantage (p=0.045 and p=0.025, respectively) (Figure 1) and also was found to have significant impact on PFS- OS in univariate regresyon analysis, we did not found HLADR negativity as an independent predictor on PFS-OS in multivariate cox regression analysis (Table 3).

Discussion

In this study, we investigated the relationship between cell surface expressions and treatment response status, PFS and OS in our own patient population. PFS and OS were found to be longer in patients expressing MPO and not expressing HLADR. However, it was shown that while MPO expression was independent of PFS and OS, HLADR was not an independent factor. Expression of CD13, CD33, CD34 and CD117 was also found to have no effect on PFS and OS. Cytogenetic risk groups were also found to be associated with PFS and OS, consistent with the literature.

The high rate of MPO expression in patients with AML has been associated with better PFS and OS with better complete response

Table 1. Descriptive features

*Age	51 (18-81)
Sex	
Female	47.8%
Male	52.2%
Risk category	
Favorable	14.2%
Intermadiate	61.1%
Adverse	24.7%
CD13 status	
Positive	90.2%
Negative	9.8%
CD33 status	
Positive	83.2%
Negative	16.8%
CD34 status	
Positive	57.6%
Negative	42.4%
CD117 status	
Positive	81.7%
Negative	18.3%
MPO status	
Positive	43.9%
Negative	56.1%
HLADR status	
Positive	69.2%
Negative	30.8%
Response rate	
CR	67.3%
NR	32.7%
Survival status	
Alive	45.1%
Ex	54.9%

*median (min-max)
min: Minimum, max: Maximum, CR: Complete response, NR: Normal response, Ex: Exitus

Table 2. Relationship of risk groups between PFS and OS

	Progression free survival						Overall survival					
Risk category	Favorable		Intermediate		Adverse		Favorable		Intermediate		Adverse	
	x^2	p	x^2	p	x^2	p	x^2	p	x^2	p	x^2	p
Favorable			4.38	.036	7.34	.007			11.13	.001	20.95	.00
Intermediate	4.38	.036			1.97	.16	11.13	.001			2.18	.09
Adverse	7.34	.007	1.97	.16			20.95	.000	2.81	0.09		

X^2: Chi-square, PFS: Progression free survival, OS: Overall survival

(CR) rates in many studies (14-17). In a study of 233 patients with AML, Dong et al. (18) demonstrated low MPO expression (<70%) as an independent risk factor for CR, OS, and PFS. They found over 70% MPO expression associated with favorable risk group, high CR rates, and longer PFS and OS (18). Kamijo et al. (19) found high MPO expression (>50%) associated with RUNX1-RUNX1T1, the KIT mutation and CEBPA double mutations, and low MPO expression (<50%) associated with DNMT3A mutation, FLT3 tyrosine kinase domain mutation and TP53 mutations. Similar to the literature, in our study, the presence of MPO expression was found to be associated with prolonged PFS and OS, and it was shown to be a favorable prognostic factor. In our study, unlike other studies, statistical significance was obtained with MPO above 20%. As mentioned above in other studies, the threshold value was determined between 50-70%. Based on these results, MPO expression at the time of diagnosis can be used as a good prognostic marker. In some places, the cytogenetics and especially the next-generation sequencer are still not easy to be reached. MPO expression can be a guide for centers that cannot reach these examinations.

Table 3. Relationship between immunophonotypic characteristics and PFS and OS, multivariate Cox regression analysis

	Progression free survival		Overall survival	
	p	Exp (B)	p	Exp (B)
CD13	0.487	0.537	0.609	0.737
CD33	0.248	0.295	0.550	1.325
CD34	0.156	0.408	0.851	0.938
CD117	0.135	2.526	0.605	0.798
MPO	0.008	7.223	0.015	2.481
HLADR	0.429	0.513	0.712	0.852

PFS: Progression free survival, OS: Overall survival

Chang et al. (20) found that CD34 positivity was associated with low CR in patients with de novo AML. And they showed that co-expression of CD34 and HLADR was a negatively independent risk factor for achieving CR (20). Other studies have shown poor response to induction chemotherapy in the presence of CD34 expression (21,22). Yang et al. (23) found that CD34 positivity was associated with low CR rates, but did not find a significant difference between CR status and CD13 and CD33 positivity.

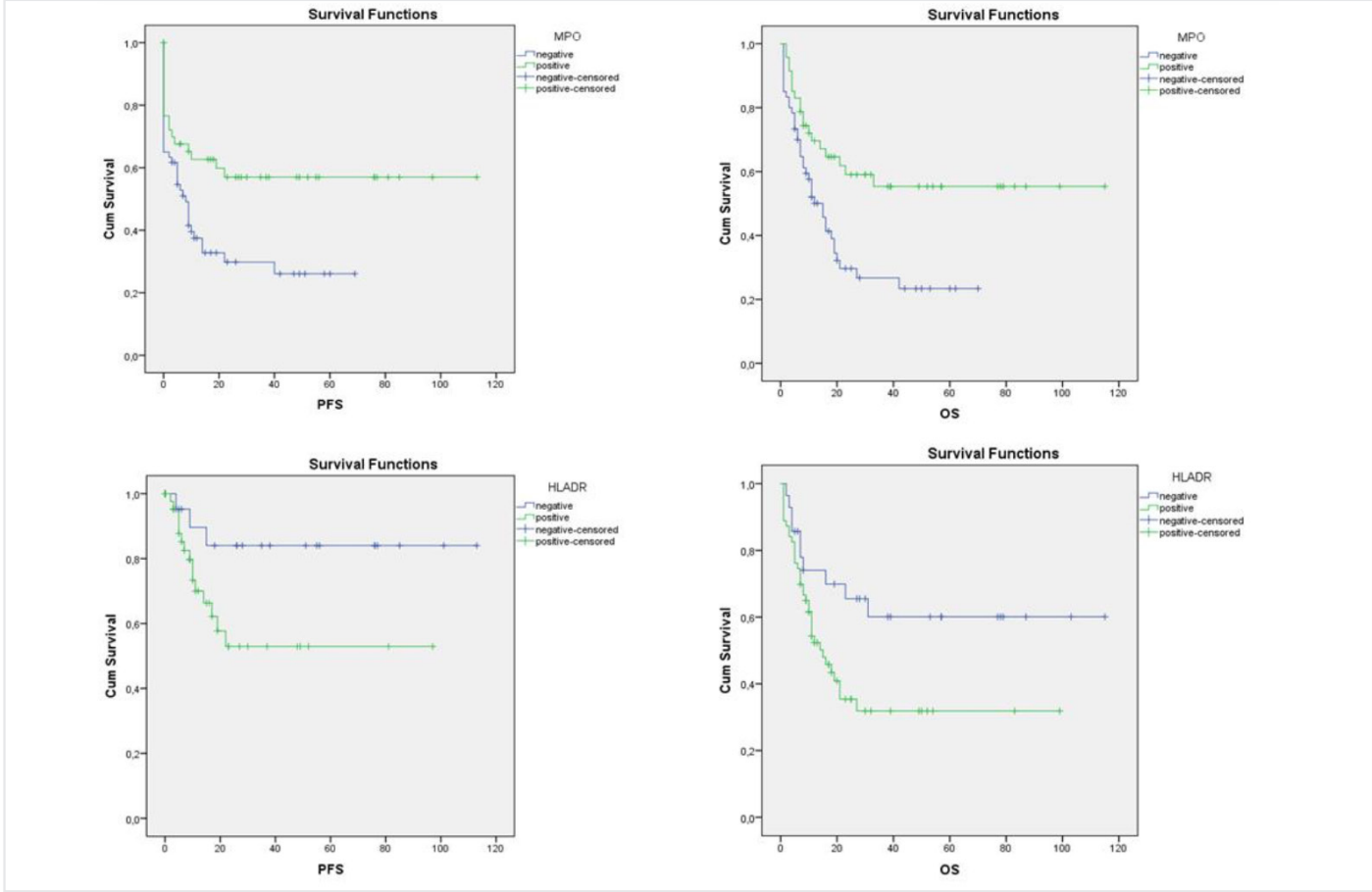

Figure 1. MPO positive patients have longer PFS and OS, HLADR negative patients have longer PFS and OS
PFS: Progression free survival, OS: Overall survival

Contrary to these data, CD34 expression did not have an effect on treatment response and survival in our study.

The absence of expression of CD9, CD11b, CD13, CD34 and CD41 in patients with AML, or the presence of CD15, CD33, CD38, CD64, and MPO expression was associated with longer OS. CD9, CD13, CD34 and CD64 have been shown to be independent risk factors on survival (24). In another study, it was found that patients in whom MPO, CD13, CD33, CD65 and CD117 were co-expressed had a better prognosis (25). Plesa et al. (26) showed that CD34-positive/CD33-positive or CD34-negative/CD33-negative was poor prognostic, CD34-positive/CD33-negative was intermediate prognostic, and CD34-negative/CD33-positive was favorable prognostic.

While several studies have shown that CD13 has no prognostic significance (9,25), another study has shown a significant correlation between the low CR rate of CD13 positivity (27). In another study, CD13 positivity was reported to be associated with low CR and short OS, whereas CD33 was not associated with CR and OS (28). CD117 was associated with decreased CR rates (29). In our study, expression of CD13, CD33 and CD117 was not related to CR, PFS and OS.

About 40-50% of patients with AML display a normal karyotype at diagnosis (30). As prognosis of AML with a normal karyotype is heterogeneous, efforts have been made to stratify patients into different risk categories and to guide therapeutic decisions. Mutations in a number of genes have been found in AML with a normal karyotype; the most commonly affected are nucleophosmin1 gene (NPM1), the FMS-like tyrosine kinase 3 gene (FLT3), and the *DNA-methyltransferase 3a* gene (DNMT3A). There are two main types of FLT3 mutations. The most common are internal tandem duplications (ITD) of different length that result in ligand-independent activation of the FLT3 receptor and a proliferative signal (31,32). The prognostic impact of FLT3-ITD is influenced by its mutational context, including the absence of the wild-type FLT3 allele (ie, homozygous or hemizygous FLT3-ITD), the concurrent mutation status of NPM1, and the FLT3 mutant/wild-type allelic ratio. Homozygous or hemizygous FLT3 and higher AR are associated with poorer outcomes (33-35). Munoz et al. (36) found that AML with FLT3-ITD often had a myelomonocytic immunophenotype expressing CD33 and CD15, with rather low CD34 expression. In addition, CD11b and CD4 were often expressed in these patients. If FLT3-ITD cannot be measured, the status of FLT3-ITD can be predicted by immunophenotypic expressions.

Study Limitations

In several studies, HLADR expression has been associated with reduced CR rates and shorter CR duration (20,37,38). In our study, it was determined that the absence of HLDR expression, which supported the literature, was associated with longer PFS and OS. Unlike the literature, no correlation was found between HLDR and response status.

Conclusion

Our aim in this study was to investigate the prognostic value of immunophenotypic markers that were routinely checked at the time of diagnosis. Previous studies have shown the prognostic significance of MPO and CD34 expression. In our study, MPO positivity was found to be consistent with longer PFS and OS, similar to the literature, but unlike the literature, no correlation was found between CD13, CD33, CD34, CD117 and PFS and OS. Randomized prospective controlled studies are needed to determine the prognostic significance of immunophenotypic features.

Ethics

Ethics Committee Approval: Van Yüzüncü Yıl University Non-Invasive Clinical Research Ethics Committee (date: 07.12.2018/ decision no: 10).

Peer-review: Externally peer reviewed.

Authorship Contributions

Concept: S.D., T.U., Design: S.D., T.U., Data Collection or Processing: Ö.E., A.D., Analysis or Interpretation: S.D., T.U., Literature Search: S.D., Writing: S.D., T.U.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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