CD11B EXPRESSION IN ACUTE MYELOID LEUKEMIA IS ASSOCIATED WITH HEMOSTATIC COMPLICATIONS AND RESPONSE TO TREATMENT

Akut Myeloid Lösemide CD11b İfadesinin Hemostatik Komplikasyonlar ve Tedaviye Yanıt ile İlişkisi

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Abstract

Aim: In our study, we aimed to investigate the effects of CD11b expression on myeloblasts on clinical course and prognosis in patients with AML.

Materials and Methods: Data of 123 patients diagnosed with AML between 2014-2017 in Trakya University Faculty of Medicine, Department of Hematology, a tertiary referral hospital in the Trakya Region, were evaluated in a retrospective manner. The diagnosis of AML was based on WHO 2016 criteria of Myeloid Neoplasms.

Results: Of the 123 patients in our study, 60 were female, and 63 were male. The mean age was 57.93 years. CD11b positivity was observed in 40 patients. Platelet counts were significantly lower in patients with CD11b positivity (p = 0.004). Likewise, D-dimer levels at presentation were higher in the CD11b positive patient group (p = 0.000). Regarding outcomes, patients with CD11b positivity were found to have lower rates of remission with first-line remission induction therapy (p = 0.003). There was no significant relationship between CD11b positivity and overall survival with Kaplan Meier survival analysis (8.5 months in CD 11b positive group, 12.1 months in negative group, p: 0.436).

Conclusion: Our study demonstrated that patients with CD11b expression had lower remission rates with remission induction chemotherapy.

Keywords: Acute myeloid leukemia, CD11b, flow cytometry, adhesion molecules, hemostasis.

Öz

Amaç: Çalışmamızda, Akut Myeloid Lösemi (AML) hastalarında CD11b ekspresyonunun klinik seyir ve hastalığın prognozu üzerine etkilerini araştırmayı amaçladık.

Materyal ve Metot: Trakya bölgesindeki bir üçüncü basamak hastane olan Trakya Üniversitesi Tıp Fakültesi, Hematoloji Anabilim Dalı'nda2014-2017 yılları arasında, Dünya Sağlık Örgütü 2016 myeloid malingniteler sınıflamasına göre AML tanısı almış 123 hastanın verileri retrospektif olarak değerlendirildi.

Bulgular: Çalışmamızdaki 123 hastanın 60'ı kadın, 63'ü erkekti. Yaş ortalaması 57.93 idi. CD11b pozitifliği 40 hastada gözlendi. CD 11b pozitifliği olan hastalarda trombosit sayısı anlamlı derecede düşüktü (p = 0.004). Aynı şekilde, D-dimer düzeyleri de CD11b pozitif hasta grubunda daha yüksekti (p = 0.000). Sonuçlar ile ilgili olarak, CD 11b pozitifliği olan hastalarda ilk remisyon indüksiyon tedavisi ile remisyon oranlarının daha düşük olduğu bulundu (p = 0.003). Kaplan Meier sağkalım analizinde; CD-11b pozitifliği ile genel sağkalım arasındabir ilişki bulunamadı (CD 11b pozitif grupta 8,5 ay, negatif grupta 12,1 ay, p: 0.436).

Sonuç: Çalışmamızda CD11b ifadesi olan hastaların remisyon indüksiyon kemoterapisi ile daha düşük remisyon oranlarına sahip olduğunu saptadık.

Anahtar Kelimeler: Akut miyeloid lösemi, CD11b, akım sitometri, adhezyon molekülleri, hemostaz.

INTRODUCTION

Acute Myeloid Leukemia (AML) is the most common type of leukemia in adults and a heterogeneous clonal disease in which the ability of the hematopoietic stem cell fails to respond to determinants of normal differentiation, proliferation, and apoptosis[1]. Interactions between AML cells and

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surrounding tissues are required for leukemic infiltration. Specific cell surface receptors mediate this biological process.

The cluster of differentiation molecule 11b (CD11b) consists of macrophage-1 antigen (Mac-1) with CD18. Various types of cells express this molecule. The CD11b expression on the surfaces of granulocytes and macrophages is increased by their activation[2]. CD11b is also an integrin family member; regulates leukocyte adhesion and migration to mediate the inflammatory response. It can also take part, chemotaxis, cytotoxicity, phagocytosis, and it is involved in the interaction of leukemic cells with the microenvironment[3].

Myeloid and non-myeloid antigens expressed on myeloblasts of acute myeloid leukemia are linked with prognosis and survival[4, 5]. Several studies have shown that CD11b expression may be related to decreased survival and poor prognosis in AML[3, 6, 7].

In our study, we aimed to investigate the effects of CD11b expression on myeloblasts on clinical course and prognosis in patients with AML, and the possibility that CD11b expression may be the explanation of the clinical variability in AML, which was attributed to the microenvironment, neutrophil activation, endothelial damage, and tissue factor release mechanisms.

MATERIAL AND METHODS

Patients: Data of 123 patients diagnosed with AML between 2014-2017 in Trakya University Faculty of Medicine, Department of Hematology, a tertiary referral hospital in the Trakya Region, were evaluated retrospectively. Acute promyelocytic leukemia (APL) patients were excluded due to the lack of CD11b expression in APL. Apart from APL we have added all AML patients whom were fit to receive standard remission induction chemotherapy. The diagnosis of AML was based on WHO 2016 criteria of Myeloid Neoplasms[8]. General features of patients including age, gender, whole blood count at diagnosis, blast percentage in bone marrow, clotting tests at presentation, response to treatment, comparison with cytogenetic evaluation, and monthly survival rates were recorded from the patient files. Patients were classified into three risk stratification groups as favorable, intermediate, and adverse according to European Leukemia Network (ELN) 2017 AML risk classification[9].

Immunophenotyping analysis: Immunophenotyping with flow cytometry was performed on bone marrow samples with Becton Dickenson (BD FACSCalibur™ platform) analyzer. Four-color flow cytometric evaluation was performed with monoclonal antibodies with fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), and peridinin chlorophyll protein (PerCP) staining. All samples were bone marrow aspiration specimens and were obtained at the time of diagnosis. After Forward and Side Scatter analysis, CD45 and Side scatter analysis, gating was performed on the blastoid area. Acute leukemia immunophenotype panel of antibodies were consisted of CD34/CD117/CD13/CD33/HLA-DR/CD10/CD3/CD7/MPO and TdT. Besides this basic panel of antibodies, CD19/CD20/CD11b/CD4/CD8 and CD16/CD56 are also evaluated. Cutoff values for all antibodies and the marker of this study, CD11b, were determined by using an absolute positive isotype control and comparing our stains with this positive control. We determined a 20% cutoff value as positive.

Treatment: The standard remission induction therapy consisted of cytosine arabinoside (ARA-C) 100 mg/m^2 continuous infusion for seven days and idarubicin 12 mg/m^2 for three days. Patients who reached a remission state received a consolidation treatment with 3 or 4 cycles of high-dose ARA-C (3 g/m2 two times a day for three consecutive days). Standard criteria were used for response definitions[10]. Bone marrow biopsies performed after remission induction chemotherapy. We did not routinely performed day 15 bone marrow biopsy instead we performed bone marrow biopsy recovery period after bone marrow aplasia. We did not use double induction regimen. If the patients is not responsive to 3+7 regimen we usually give FLAG based salvage regimen[11]. Patients were referred to allogeneic hematopoietic stem cell transplantation (allo-HSCT) from a full matched HLA donor and if they are fit to tolerate.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standard. Informed consent was obtained from all individual participants included in the study. Trakya University Ethical Committee Approval: 2018/120 Date: 02.04.2018.

Statistical Analysis: Statistical analysis performed with SPSS 20.0. Normality distributions of all variables were tested by the Shapiro-Wilk test. Normalized data were used for further analysis. Parametric variables compared with the Chi-Square test, nonparametric variables, analyzed with the Mann-Whitney U test, and Kaplan-Meier

analysis performed for survival. A two-sided p-value less than or equal to 0.05 was considered of statistical significance.

RESULTS

Patient Characteristics
Of the 123 patients in our study, 60 were female, and 63 were male. The mean age was 57.93 (20-95). The demographic data of the patients are summarized in Table 1. CD11b positivity was observed in 40 patients.

Table 1. Demographic data and characteristics of the patients

Age (Year)	57,93 ±17,36 (20-95)
Gender	
Male	60 (48.8%)
Female	63 (51.2%)
Number of patients CD11b positive	40 (31.5%)
Number of Patiens CD 11b negative	83 (68.5%)
Patients according to ELN Risk Stratification	
Favorable	28 (22.8 %)
Intermediate	58 (47.1 %)
Advers	37 (30.1%
Mean Hemoglobin (gr/dl)	9,28 gr/dl ± 5,77 gr/dl
Mean leukocyte count x10^9/L	34107,95 x10^9/L ± 44037,36 x10^9/L
Mean Thrombocyte count x10^9/L	58254,21 x10^9/L ± 73468,87 x10^9/L

Mean leucocyte count at presentation was 34107.95 x10^9/L, mean hemoglobin level 9.28 g/dL, and the mean platelet count was 58254.21 x10^9/L. Complete remission obtained in 61 (49.5 %) of the patients with first induction chemotherapy. Fifteen patients (11.4 %) could proceed to the Allo-HSCT. Induction mortality is seen in 21 (17.7 %) patients

Comparisons
There was no significant relationship between CD11b positivity and ELN genetic risk stratification, age, sex, hemoglobin level, leukocyte count, blast percentage in the bone marrow, and genetic markers (p values>0.05). The platelet counts were significantly lower in the CD11b positive group (p = 0.004). Similarly, the D-dimer levels at presentation were higher

in the CD11b positive group (p = 0.000). Patients with CD11b positivity were found to have lower rates of remission with first-line remission induction therapy (p = 0.003) (Table 2 and Table 3).

Table 2. Relationship with CD11b positivity

	CD11b positive patients (n:40) (%)	CD11b Negative patients (n:83) (%)	P value
Remission with first induction chemotherapy	9/40(22,5)	52/83 (%62,6)	**0,003**
Sex	17 female 23 male	43 female 40 male	0,344
Age	59,30	57,27	0,545
Hemoglobin (gr/dl)	9,03 ± 1,93	9,41 ± 6,91	0,730
Leucocyte count x10^9/L	33792,00 ± 36661,47	32252,83 ± 48716,43	0,530
Thrombocyte count x10^9/L	31427,50 ± 24608,04	71183,01 ± 64984,02	**0,004**
D-dimer (ng/mL)	8,87 ± 6,44	4,25±5,28	**0,000**
Overall survival (months)	8,5	12,1	0,436

Table 3. Relationship with CD11b positivity and ELN Risk Stratification

	ELN Favorable (n) (%)	ELN Intermediate (n) (%)	ELN Adverse (n) (%)	P value
CD11b Negative	23	34	26	0,084
CD11 b Positive	5	24	11	
Total	28	58	37	

Our study was an observational retrospective study. Median duration of follow-up was 18.4 months (0-48). Mean overall survival of the all cohort was 23.5 ± 1.85 months. There was no significant relationship between CD11b positivity and overall survival (8.5 months in CD11b positive group, 12.1 months in negative group, p: 0.436).

DISCUSSION

AML has distinct expressions with immunophenotyping, and also aberrant leukocyte surface antigens can be found[7]. Particular surface or intracellular markers are observed on myeloblasts however aberrant antigens which are not specific for AML myeloblasts could be expressed or loss[5]. These abnormal expressions or decrease of

expression are usually not associated with specific lineage or cell type. On the other hand, aberrant antigens can be used for definition of leukemia associated immunophenotype, which could serve a useful tool for future minimal residual disease analysis[12].

CD11b is critical in cellular adhesion and migration, and a surrogate marker for neutrophil activation[13,14]. Integrins, which include CD11b, are demonstrated to be involved in tumor resistance, cellular interactions, and microenvironment involvement[15,16]. In invasion and metastasis of solid tumors, CD11b positive cells have shown to act on antigen-presenting cells and enhance the expression of PD-L1[17, 18]. Therefore, integrins and CD11b are considered as potential targets. Although the disease biology and clinical course in AML are quite heterogeneous, interactions of hemopoietic progenitor cells, endothelial cells, and stromal cells are becoming important. In myeloproliferative neoplasms, which are chronic diseases of myeloid progenitor cells, expression of CD11b on granulocytes was demonstrated to increase the hypercoagulability and monocyte-derived tissue factor (m-TF), by causing neutrophil chemotaxis and endothelial damage[19, 20]. In AML, which is the acute form of proliferative disease of myeloid progenitor cells, we observed that this integrin expression might result in hypercoagulation, coagulopathy, or at least disturbances in hemostatic status.

Leukocytes expressing CD11b on the surface were able to recognize and interact with fibrinogen, and it was shown that fibrinogen enhances leukocyte binding to endothelial cells in vitro, with simultaneous interaction with CD11b/CD18 on leukocyte and intercellular adhesion molecule-1 (ICAM-1)[21]. Although we could not demonstrate the association of

cytogenetic risk groups with this expression, assessment of CD11b expression in AML patients transformed from myeloproliferative diseases may particularly support this connection.

CD11b is required for the interaction of leukemic cells in the bone marrow microenvironment. It also suppresses the immune system and is a marker for myeloid-derived suppressor cells that play a role in the progression of malignancies[22, 23]. Therefore, we suggest that CD11b expression can be used as a prognostic marker in AML. CD11b expression in AML was found to be associated with lower survival in several studies. The poor prognosis of CD11b was demonstrated in 382 AML patients in Eastern Cooperative Oncology Group's study[24]. In another study, data of the 48 AML patients were evaluated. CD11b expression was associated with poor prognosis, and patients with poor cytogenetic risk groups had higher CD11b expression[2]. However, we could not find an association with cytogenetic risk classification with CD11b expression in our study. In another retrospective analysis, CD11b expression was found as a prognostic factor for lower complete remission rates in multivariate analysis[6]. In a meta-analysis which was evaluated 13 studies including 2619 patients; the expression of CD11b was associated with lower complete remission and overall survival rates[3]. Our study with limited data showed similar results with this meta-analysis. CD11b expression (leukocyte activation) might contribute to the deterioration of hemostasis tests and coagulopathy by activation of FXa. It was shown that activated monocytes activate binding to Factor X by expressing CD11b/CD18, and they perform prothrombin thrombin transformation[25]. This finding might be

a clue of altered hemostasis and coagulation tests we found in CD11b positive AML patients.

The fact that our study was designed in a retrospective manner, our sample size was relatively small and limited follow-up period could be considered as three limitations of our study. However, our findings may be a guide for CD11b expression in AML patients.

CONCLUSION

We observed that AMLpatients with CD11b expression had lower remission rates with first-line remission induction chemotherapy. We thought that this finding should be kept in mind while deciding the treatment of CD11b positive AML patients.

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