Clinical Significance of CD87 Expression in Acute Myeloblastic Leukemia

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ABSTRACT

CD87 or uPAR (urokinase type plasminogen activator receptor) expression leads to conversion of plasminogen to plasmin to mediate extravasation by proteolytic breakdown of adhesion molecules responsible from cell contact. uPA (urokinase type plasminogen activator) system consists of a proteinase, its receptor uPAR, and two inhibitors (plasminogen activator inhibitor [PAI] 1 and 2). CD87 expression may have implications for diagnosing acute myeloblastic leukemia (AML) and determining its prognosis, as well as for follow-up of minimal residual disease (MRD). In this study we determined CD87 levels in bone marrow or peripheral blood immunophenotypically and investigated its relationship with some prognostic factors in 35 newly diagnosed and untreated AML patients. While there was no significant correlation between CD87 expression and age, sex, admission white cell count, and CD34 expression, a significant relationship was found between CD87 expression and organomegaly. This relationship was prominent in cases with AML showing monocytoid differentiation. No correlation was found between CD87 expression and treatment response. In AML patients hepatosplenomegaly and mucocutaneous infiltrations are associated with increased CD87 expression. Extramedullary extension is common in leukemias with monocytoid differentiation. uPA, by binding to CD87, initiates conversion of plasminogen into plasmin to mediate extravasation of cells through endothelium by proteolytic breakdown of endothelium-associated adhesion molecules. It plays role in adhesion, migration and metastasis of leukemia cells. This condition may also explain the relationship between CD87 and the rate of organomegaly. It may prove a useful marker for follow-up of MRD when detected at the time of diagnosis.

Keywords: Acute Myeloblastic Leukemia, Flow Cytometry, CD87

ÖZET

Akut Miyeloblastik Lösemide CD87 Ekspresyonunun Klinik Önemi

CD87 veya uPAR (ürokinaz tip plazminojen aktivatör reseptörü) ekspresyonu plazminojenin plazmine çevrilmesini sağlayarak hücre temasından sorumlu adezyon moleküllerinin proteolitik parçalanması ile ekstravazasyona aracılık etmektedir. uPA (ürokinaz tip plazminojen aktivatör) sistem; bir proteinaz ve onun reseptörü olarak görev yapan uPAR ve iki inhibitörden (plazminojen aktivatör inhibitör [PAI] 1 ve 2) oluşur. CD87 ekspresyonu akut myeloblastik lösemi (AML)de tanıda, prognozda ve minimal rezidüel hastalık (MRH) takibinde önemli olabilir. Bu çalışmada yeni tanı almış ve tedavi verilmemiş 35 AML hastasında kemik iliği veya periferik kanda immunfenotipik olarak CD87 düzeylerini ve bazı prognostik faktörlerle ilişkisini değerlendirdik. CD87 ekspresyonu ile yaş, cinsiyet, başvurudaki beyaz küre sayısı, CD34 ekspresyonu arasında anlamlı bir ilişki saptanmazken, organomegali ile istatistiksel olarak anlamlı ilişki saptandı ve bu monositoid farklılaşma gösteren AML olgularında belirgindi. Tedaviye cevap açısından CD87 ekspresyonu ile korelasyon bulunmadı. AML hastalarında hepatosplenomagali ve mukokutanöz infiltrasyonlar CD87'nin artmış ekspresyonu ile ilişkilidir. Monositoid farklılaşma gösteren lösemilerde ekstramedüller yayılım sıktır. CD87'ye bağlanan uPA, plazminojenin plazmine dönüşümünü başlatarak endotel ilişkili adezyon moleküllerinin proteolitik olarak ayrılması ile endotel boyunca hücrelerin damar dışına çıkmasına aracılık eder. Lösemik hücrelerin adezyon, migrasyon ve metastazında rol oynar. Bu durum CD87'nin organomegali sıklığı ile ilişkisini de açıklayabilir. Tanı anında saptanması ile hastalarda MRH takibinde yararlı bir belirteç olabilir.

Anahtar Kelimeler: Akut Miyeloblastik Lösemi, Akım Sitometri, CD87

INTRODUCTION

AML is a clonal hematological, heterogenous group of diseases caused by the maturation defect and the uncontrolled proliferation of myelocytic, erythrocytic, and megakaryocytic cell series of bone marrow. These malignant cells stop the normal development and the maturation of erythroid, myeloid, and megakaryocytic precursors. The lifespan, proliferation and differentiation of hematopoietic cells are regulated by cytokines. uPA system consists of a proteinase, its receptor (uPAR or CD87), and two major inhibitors (PAI 1 and PAI 2).[1,2] The plasminogen activation is considered to play a central role in the regulation of pericellular proteolysis under both the normal and pathological conditions. Functions of uPA-uPAR are regulated by PAI 1 and 2. Cellular functions related to uPAR have multiple regulatory functions in cell migration, leucocyte adhesion, chemotaxis and signal conduction.[1-4]

uPA and uPAR are expressed by leucocytes (neutrophil leucocytes, monocytes, macrophages, eosinophils, activated lymphocytes), endothelial cells and fibroblasts. Among the normal bone marrow cells uPAR is expressed on some myeloid precursors (promyelocytes, myelocytes, and metamyelocytes), monocytes, and their precursors but not on CD34(+) hematopoietic stem and progenitor cells.[1,5] Their functions in healthy cells include the conversion of plasminogen into plasmin, the extravasation associated with proteolytic breakdown of the adhesion molecules which are responsible from cell contact, the regulation of cytoskeleton-dependent events such as mobility and degranulation and increased bleeding tendency.[6-10]

In clonal hematopoietic disorders, CD87 expression studies have focused on acute leukemias and multiple myeloma. We studied CD87 expression frequency with flow cytometry method in patients with AML. The relationship between CD87 expression and the factors including age, admission white blood cell count, and complete remission (CR) rate were also investigated.

PATIENTS AND METHODS

This study included 35 newly diagnosed cases of AML. AML diagnosis and classification were made using the clinical findings, the peripheral smear, the morphological and histochemical examination and the immunophenotyping of bone marrow or peripheral blood. Patients were divided into subgroups according to French–American–British (FAB) classification. According to this, 14 patients were AML-M0-M1, 12 were M2, 4 were M3, 3 were M4 and 2 were M5. Thirty-two patients had primary AML while 3 had secondary AML. Informed consent was taken from all patients in accordance with the Declaration of Helsinki.

Bone marrow or peripheral blood sample was used for the flow cytometric study. CD3, CD5, CD7, CD10, CD11b, CD13, CD14, CD15, CD19, CD20, CD33, CD34, CD56, CD64, CD117, Anti HLA DR, MPO, TdT, CD45, CD87 expression were studied. The following techniques were used for flow cytometric study:

- Ethylenediaminetetraacetic acid (EDTA) tubes were used for fresh blood and bone marrow samples, and polystyrene tubes were used for test samples.
- Monoclonal antibodies (MoAb) labeled with fluorescent stain (FITC, PE, perCP) were used.
- Bone marrow samples were diluted with PBS (Phosphate Buffered Saline) while the direct study of peripheral blood was made.
- Peripheral blood and bone marrow samples of 100 μL were put into polystyrene tubes. MoAb of 20 μL was added to each sample. CD45/CD14 and G1FITC/G2aPE isotypic control were used for each study. Upon adding the antibodies, tubes were vortexed at low speed for 3 seconds, and incubated at dark and room temperature for 15-30 minutes.
- For the removal of erythrocytes form, the medium 2 mL of FACS-Lysing solution was added to each tube. The tubes were then vortexed at low speed for 3 seconds, and incubated at dark and room temperature for 10 minutes. After incubation, the tubes were centrifuged at room temperature for 5 minutes. After centrifugation, the supernatant was aspirated. The 50 μL cell suspension at the bottom was vortexed at low speed. Two ml PBS was added to tubes followed by vortexing at low speed for 3 seconds and re-centrifuging at room temperature. The 50 μL cell suspension at the bottom was vortexed at low speed and 500 μL PBS was added on top of it.

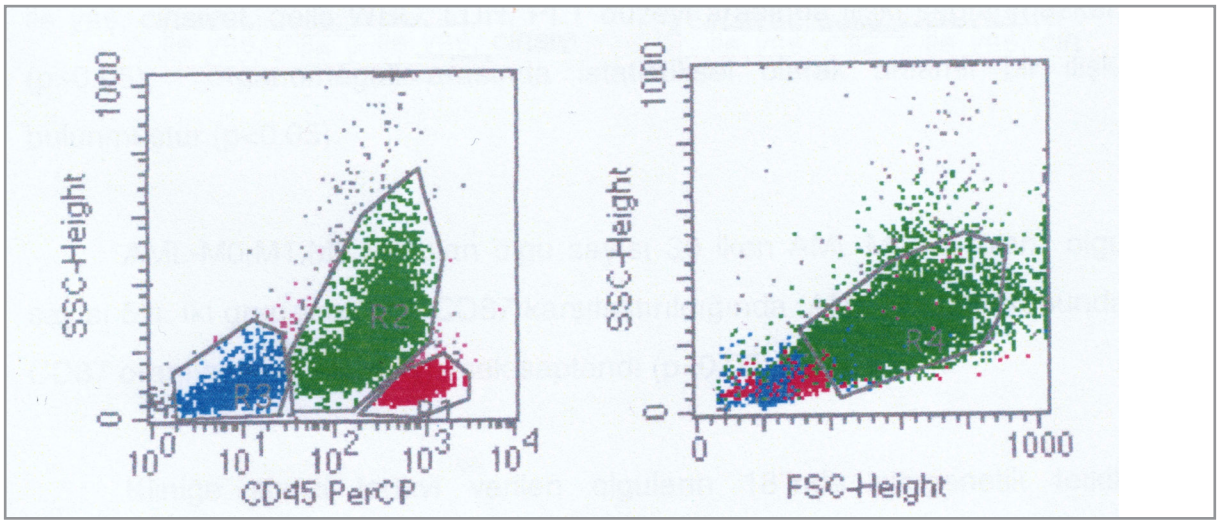

Figure 1. Selection of blast cells by flow cytometry

- The cells were covered with aluminium foil and kept at dark at 2-8^{0}C until the analysis.
- "Acquisition" process of MoAb-labeled cells was performed in FACS Calibur (Becton-Dickinson) device. The samples were gated according to CD45 SSC (Side Scatter) in order to evaluate the blasts in Cell quest program (Figure 1).
- Combinations were used for the multiparametric analysis (Figure 2).
- An expression percent > 20% was considered positive.

Five of 35 patients diagnosed with AML left the study for the follow-up at another center. Seven patients died prior to the therapy. Four patients received only supportive therapy because of the advanced age. Sixteen patients were given remission induction therapy composed of cytosine arabinoside and anthracyclin (daunorubicin, mitoxantrone, or idarubicin) whereas one patient was administered a remission induction therapy consisting of cytosine arabinoside and etoposide due to a preexisting cardiac problem despite the young age. Two patients diagnosed with AML-M3 were given idarubicin and all-trans retinoic acid(ATRA) remission induction therapy. Eighteen of patients admitted for remission induction therapy gave peripheral blood or bone marrow samples for conventional cytogenetic study as well as t(8;21), t(15;17) and inv(16) study.

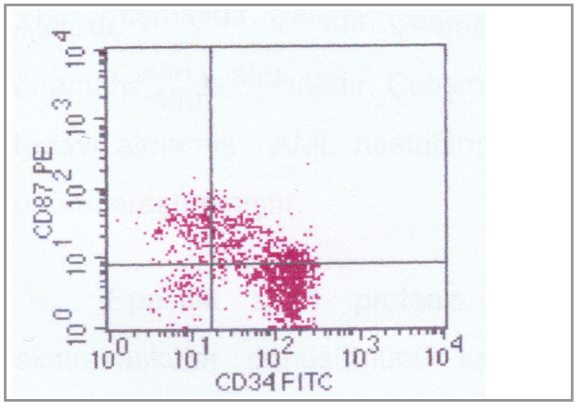

Figure 2. CD87, CD34 positivity in blast cells

A thrombocyte count of > 100000/μL, a neutrophil count of > 1000/μL, and a blast percentage < 5% in bone marrow following the therapy were considered as CR.

Statistical Analysis

The study data were analyzed using SPSS 11.5 software package. Continuous variables were presented as mean ± standard deviation or median (min-max) and categorical variables were presented as number of observations and %. The differences in median CD87 expression between groups were compared by Mann Whitney U test. Degrees of associations between continuous variables were calculated by Pearson's product-moment or Spearman's Rank correlation coefficients, where appli-

Table 1. General patient characteristics

Age [years, median/(min-max)]	53 (18-77)
Sex (F/M)	15/20
WBC [µL, median/(min-max)]	28100 (310-457000)
Hb [gr/dl, mean±SD]	8.1±2.0
Plt [µl, median/(min-max)]	35000 (7000-520000)
LDH [U/L, median/(min-max)]	352 (195-2477)
FAB subtypes	
M0-M1	14 (40.0%)
M2	12 (34.3%)
M3	4 (11.4%)
M4	3 (8.6%)
M5	2 (5.7%)
CD34 positivity	26 (74.3%)
Organomegaly (lymphadenomegaly/hepatomegaly/splenomegaly)	13 (37.1%)
Cytogenetics	
Favourable	5
Intermediate	11
Unfavourable	2
CR	11 (57.9%)

cable. A p value less than 0.05 was considered statistically significant.

RESULTS

Demographic data (age, sex), leucocyte (WBC) and thrombocyte counts, hemoglobin (Hb) level and lactate dehydrogenase (LDH) levels, FAB subtypes, cytogenetic properties, CR ratio, and CD34 and CD87 expression are given in Table1.

Twelve patients were older than sixty years while twentythree patients were younger than sixty years. Twenty-two patients had a WBC count greater than 25000/μL. Thirty-two patients had primary AML while three had secondary AML (two patients had conversion from myelodysplastic syndrome and one from chronic myelocytic leukemia). As the number of the secondary cases was so low, no statistical comparison could be made between the primary and secondary AML with regard to CD87 positivity.

The relationship of CD87 expression with age, WBC and PLT counts, LDH levels, and CD34 expression was studied. The results are presented in Table 2. According to this table, there was no statistically significant correlation (p> 0.05). The association of CD87 expression with sex, presence of organomegaly and CR ratio was analyzed in Table 3. While, there was no statistically significant difference in CD87 expression regarding the sex and CR, whereas CD87 expression in organomegaly positive group was higher than the negative one (p= 0.045).

Table 2. Correlation between CD87 expression and properties of AML patients

	Correlation Coefficient	p-value
Age	0.312	0.068
WBC	-0.017	0.921
Hb	0.105	0.547
PLT	-0.300	0.080
LDH	-0.087	0.619
CD34	-0.293	0.087

Thirty patients had AML-M0, M1, M2, and M3 and 5 patients had AML-M4 and M5. AML-M4, M5 group had a significantly higher CD87 expression ratio [median: 45.0 (min: 11.0-max: 67.0)] than AML-M0, M1, M2 [median: 2.5 (min: 1.0-max: 37.0)] group (p< 0.001).

A cytogenetic testing could be performed in eighteen patients who are given therapy. Five of AML cases had favorable cytogenetical properties (two patients had t(15;17), two had t(8;21), and one had inv(16)), two patients had unfavorable cytogenetical properties (one patient had complex karyotype and one had 11q23 anomaly), and eleven patients had intermediate cytogenetical properties. Due to the low number of subjects no statistical analysis could be performed between CD87 expression and the the cytogenetical properties.

Eleven (57.89%) of nineteen AML cases who received remission induction therapy achieved CR at the end of 4 weeks. No significant association was present between CR rates and CD87 expression (p= 0.351). One patient died as a consequence of disseminated intravascular coagulation (DIC) during the therapy. Two patients who deceased due to DIC during therapy and due to intracranial bleeding prior to therapy had a CD87 expression lower than 20%.

A cut-off-level of 20% (positivity> 20%) for CD87 yielded only five patients with CD87 positivity. This low number was attributed to the low number of patients with AML-M4-M5.

Table 3. Association between CD87 expression with sex, organomegaly and CR ratio

	CD87 expression median (min-max)	p-value
Sex		0.496
Male	4.5 (1.0-50.0)	
Female	2.0 (1.0-67.0)	
Organomegaly		0.045
No	2.5 (1.0-37.0)	
Yes	10.0 (1.0-67.0)	
CR		0.351
No	8.0 (1.0-50.0)	
Yes	3.0 (1.0-19.0)	

No significant difference was found between patients older than 60 [median: 4.0 (min: 1.0-max: 67.0)] and younger than 60 [median: 3.0 (min: 1.0-max: 50.0)] with regard to CD87 expression (p= 0.719).

DISCUSSION

The survival rates for AML continue to be poor despite the recent scientific advances. Remission induction therapies achieve a CR rate of approximately 70% while 50% of cases have relapses. Thus, only 20-30% of cases achieve a survival longer than 10 years. Many prognostic parameters have been studied in AML. The importance of immunophenotypic properties remains debatable.

Proliferation, differentiation and survival of hematopoietic cells are regulated by hematopoietic cytokines. Urokinase as a specific serin protease catalyses extravascular conversion of plasminogen into plasmin. In this way, plasmin as a protease degrading extracellular matrix proteins and adhesion molecules, mediates invasion, metastasis, mobility, and degranulation of tumor cells. CD87 is stored in the intracellular vesicles and expressed on the cell surface following activation. Increased CD87 activation leads to the consumption of plasminogen inhibitors and increases bleeding complications.[1]

CD87 is expressed particularly by AML subtypes showing monocytoid differentiation.

The close relationship between the monocytic group and CD87 may depend on the stage-specific feature of the late myelomonocytic cells. The lack of CD87 in most of the bone marrow neutrophiles can be explained by the homing characteristics of the specific subgroup.

Knapp et al for the first time reported a CD87 expression rate of 41% using VIM5MoAb CD87 in a 50-case series of acute leukemia patients.[11] AML subgroup showing monocytic differentiation (FAB AML-M4, M5) showed the most frequent expression. A weak expression was observed in the B cell acute lymphoblastic leukemia (ALL) cases. Plesner et al., using two different MoA, reported similar CD87 expression in normal monocytes and myeloblasts.[6] A recent study by Atfy et al. reported the highest expression rate for AML-M4, M5 and the lowest expression rate for AML-M0 subtypes.[12] Lanza et al. demonstrated that CD87 expression showed a heterogenous pattern associated with the maturation level of myeloblasts and the reactivity depending on the associated cell (granulocytic or monocytic).[5] In that study, CD87 expression was found low in undifferentiated AML while a higher expression was observed in monoblastic AML. Sixty of all 74 cases with AML showed positivity. CD87 positivity was observed in only 3 of 24 ALL cases, and the extent of the positivity was less than that of AML cases. Mustjoki et al. also reported similar findings.[13] In this study, despite the low number of patients, a significantly higher rate of CD87 positivity has been detected in AML M4-M5 group. However, Jardi et al., by using MoAb, reported that only a minority of AML patients showed surface uPAR expression (positive if > 20%).[14]

uPA-uPAR complex is considered to contribute to invasive and migratory properties of leukemic cells. Leukemic cells tend to extravasate, which is a condition thought to be mediated by uPA found on the surface of leukemic cells. Blood brain barrier is an important barrier for foreign cell entry into central nervous system but leukemic cells and solid tumors are able to overcome it via the same mechanism.[1,4] Extramedullary extension is common in leukemias showing monocytoid differentiation. Bound to uPAR, uPA initiates the conversion of plasminogen into plasmin, and this mechanism mediates the extravasation of cells across the whole endothelium by the proteolytic cleavage

of endothelium-associated adhesion molecules. It plays a role in adhesion, migration, and metastasis of leukemic cells. It may also explain the relationship between CD87 and the frequency of organomegaly.[1,7] A significant relationship between CD87 and organomegaly was also found ($p< 0.05$).

CD34 is expressed by AML patients at various rates. Some studies have shown that CR ratio is lower, the relapse rate is higher, and CD34 portends a poor prognosis in CD34 (+) AML patients. CD34 positivity has also been detected in the group with favorable prognosis.[15] Although no significant correlation could be detected between CD34 and CD87 expression, the majority of CD87 positive patients were also CD34 positive. Low number of patients could be the reason for this unsignificant correlation. Since co-expression of CD87 and CD34 does not present on normal hematopoietic cells, the presence of such a combination may be used for the detection of leukemic cells in patients in remission, during the follow-up period of residual disease.[5,7]

Graf et al. showed the relationship between CR and CD87 and reported a lower remission rate and a shorter disease-free survival in patients with AML expressing CD87 at a high degree. It was emphasized that its expression was more prominent in leukemias with monocytoid differentiation and associated with poor prognosis.[7] Some other studies have also reported similar results.[13] We could not detect any relationship between CD87 and CR since, in our opinion, the number of monocytoid AML cases was low for an accurate statistical analysis.

Various studies investigating CD87 expression with cytogenetic studies have reported different results. While some studies have reported an increased incidence of chromosome anomalies with higher CD87 expression, some others have refused such a correlation.[5,7] In the study by Graf et al. no significant difference regarding CD87 expression was found among the cytogenetic risk groups.[7] In our study a cytogenetic study could be performed in only eighteen of patients who received therapy. Because of the low number of cases, statistical analysis of the relationship between CD87 expression and cytogenetic properties was not evaluated.

An increased CD87 expression in leukemic patients leads to conditions of an increased rate of the consumption of plasma inhibitors and accelerated fibrinolysis, both of which are responsible for bleeding complications.[5-7,11] Tissue factor and CD87 are the key cellular receptors triggering coagulation and fibrinolysis. Among the factors responsible from bleeding in leukemia are abnormal fibrinolytic response and/or abnormal expression of tissue factor by blasts. uPA-uPAR interaction induces plasminogen activation on cell surface, leading to a wide-spectrum proteolysis. In this way, an increased CD87 expression in leukemic cells may result in a hyperfibrinolytic tendency.[16] This CD87 expression may lead to a more aggressive disease. Bleeding complications may take place prior to and during the therapy. PAI 1 and 2 are responsible form regulation of this hyperfibrinolytic state but their amount is decreased by increased uPA system activity. Previous studies have shown that increased levels of PAI 2 are associated with favourable prognosis.[13] In our study both of two patients who died because of DIC during the therapy and the intracranial bleeding prior to the therapy had a CD87 expression above 20%.

In conclusion, this study has shown a more frequent CD87 positivity in AML with a monocytoid differentiation, the relationship between the CD87 positivity and the organomegaly, and its likely relation with the bleeding complications. However, extensive studies including the cytogenetic characteristics are necessary in order to emphasize the clinical and prognostic importance of CD87.

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