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# Significance of CD49f in diagnosis of minimal residual disease in pediatric acute leukemia

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**Abstract**

Minimal residual disease (MRD) is the most important prognostic indicator in acute lymphoblastic leukemia (ALL) in childhood. Multiparametric flow cytometry (FCM) is a technique that is often used to determine MRD, and many markers have been identified. Another marker examined in the MRD analysis is CD49f. We aimed to determine the importance of CD49f expression in MRD detection. Immunophenotyping MRD and CD49f expressions were performed in patients with Pre-B cell ALL at the diagnosis, and on the day 15. 27 patients were included (F/M: 10/17). The mean age was 6.6±4.8 years. 6 (22.2%) patients were in the standard risk group, 14 (51.9%) patients were in the intermediate risk group, and 7 (25.9%) patients were in the high-risk group. MRD was detected in 15 (55.6%) patients. Cytomorphological remission was observed in 21 (77.7%) patients on the 15th day. 10 of these patients (66.6%) were MRD positive. CD49f levels at diagnosis and at 15$^{th}$ day were mean 38.4 ± 22.1 and 5.4±12.6, respectively. A significant decrease in CD49f expression was observed at follow up (p=0.00). Mean CD49f levels in MRD positive and MRD negative patients were 7.8±17 and 2.8±2 at day 15, respectively (p=0.64). There was no correlation between MRD and CD49f at day 15 (p=0.54). We observed that leukemic blasts express CD49f at a high rate, and this expression continues to decrease on the 15$^{th}$ day. We concluded that studies including more patients are required to assess the performance and importance of CD49f as an indicator in MRD.

**Keywords:** acute lymphoblastic leukemia, minimal residual disease, CD49f

## 1. Introduction

Acute lymphoblastic leukemia (ALL) is the most prevalent hematological malignancy in childhood (1). Understanding the clinical, immunological, and cytogenetic characteristics of the disease has highlighted the importance of disease risk categorization and risk-directed treatment (2). The prognostic risk factors are/include clinical presentation characteristics, genetic subtype, germline cancer predisposition, and minimal residual disease (MRD) (3). Early response to initial treatment has been demonstrated to be one of the important determinants of the outcome (4). Poor morphological response in the first month of treatment has been accepted as a poor prognosis indicator. However, morphological features were found to be insufficient, and more sensitive techniques were required to be developed in the assessment of remission (5). MRD can detect $10^{-3}$-$10^{-6}$ leukemic blasts. Therefore, MRD significantly reflects the response to treatment and serving as a good predictor (6).

In MRD studies, multiparametric flow cytometry (FCM), polymerase chain reaction, and next-generation sequencing methods are utilized (7). Immunophenotyping with FCM in MRD determination is fast, sensitive, and simple to use in most cases (7). Many studies have shown that combinations of CD10, CD20, CD22, CD19, CD34, CD38, CD45, and CD58 can be employed for MRD assessment (8). CD49f is an adhesion molecule expressed on T cells, monocytes, platelets, epithelial, endothelial cells, and perineural cells. Many studies have shown that CD49f is overexpressed on days 19 and 46 of induction therapy (9). However, the role of CD49f in MRD studies is still unexplained.

This study aimed to determine the importance of CD49f expression and its compatibility with FCM in the MRD detection with pre-B ALL patients.

## 2. Matherials and Methods

### 2.1. Patients

The study was conducted in the Pediatric Hematology and Oncology Clinic, Erciyes University Hospital between January 2012 and January 2013. All children newly diagnosed with B-ALL and treated under the Turkish Acute Lymphoblastic Leukemia Berlin Frankfurt Münster 2000 (TR-ALL BFM 2000) protocol were eligible for the study and included based on informed consent (10). Disease risk categories, demographic, and laboratory data were recorded from patient files. Risk groups were formed based on the clinical and laboratory findings of TR-ALL BFM criteria.

Immunophenotypic MRD was evaluated using monoclonal

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antibodies such as Cyto 16, CD45, CD19, CD20, CD10, CD22, and CD58 on 300000/mm$^3$ cells on day 15 using the Beckman Coulter FC500 device. Leukemic blasts containing more than 0.01% of mononuclear cells were regarded as positive for MRD (+).

CD49f expression was examined at the time of diagnosis and on the 15$^{th}$ day. Additionally, MRD and CD49f were assessed again and correlated on the 15th day.

The study was approved by the Erciyes University Ethics Committee and supported by Erciyes University Scientific Research Projects Coordination Unit (TSU-12-3805).

### 2.2. Studying CD49f with FCM

100 µL of filtered blood sample was taken and 5 µL of CD19 PC7, CD10 FITC, and CD49f PE moAbs were added to it and incubated for 10 minutes in the dark. After incubation, erythrocytes were lysed, leukocytes were stabilized, and cell membranes were fixed using immunoprep "Coulter" lysing reagents. 2 mL of isoflow was added and washed twice for 5 minutes at 1200 rpm. The pellet was poured out, and 1 mL isoflow was added to the tube and processed on the Beckman Coulter FC500 device. The analysis was performed using CXP software. Fig. 1-2 shows the CD49f examination of one patient.

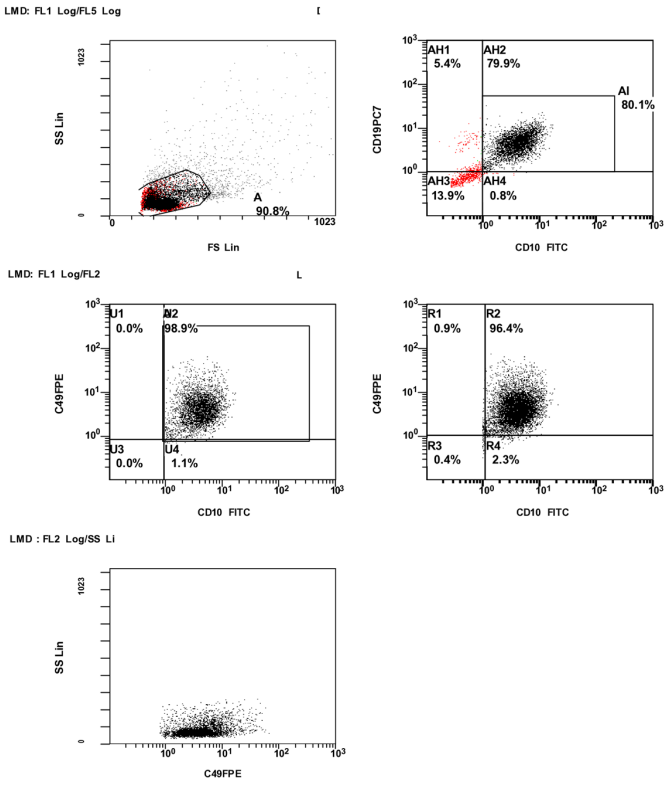


**Fig. 1.** CD49f analysis illustration in diagnosis

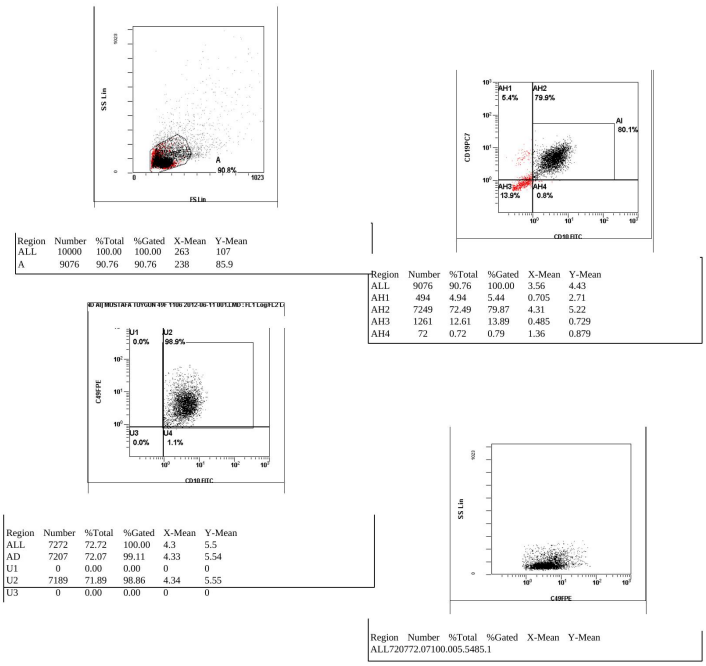


**Fig.2.** CD49f analysis illustration in diagnosis

### 2.3. Statistical Analysis

IBM SPSS Statistics for Windows, version 25 (SPSS Inc, Chicago, IL, USA) was used to perform the statistical analysis. The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov /Shapiro-Wilk's test) to determine whether they are normally distributed. Normally distributed data were expressed as mean±standard deviation, while non-normally distributed data were expressed as median [minimum-maximum]. The student's t-test was used for pairwise comparisons of normally distributed variables, and the Mann-Whitney U test was used for pairwise comparisons of non-normally distributed variables. Kruskal-Wallis test and Chi-square test were used to compare data from more than two groups. *p*-value <0.05 was considered significant.

## 3. Results

### 3.1. Patient characteristics

Twenty-seven patients were included in the study, of whom 10 (37%) were female. The mean age was 6.6±4.8 years. According to the risk classification used in the TR-ALL BFM treatment protocol, 6 (22.2%) patients were in the standard risk group (SR), 14 (51.9%) patients were in the intermediate risk group (IR), and 7 (25.9%) patients were in the high-risk group (HR). There was no central nervous system (CNS) involvement except for 1 (3.7%) patient in the high-risk group. BCR-ABL1 gene mutation was found in 1 (3.7%) patient in the genetic examination.

The median leukocyte, absolute neutrophil count, platelet count and, hemoglobin at the time of diagnosis were 6090/mm$^3$ (1720-215,000), 730/mm$^3$ (30-8500), 6.7 g/L (1,4-11,2), and 78,000/mm$^3$ (8000-818,000), respectively.

In the peripheral smear on the eighth day, a corticosteroid response was observed in 22 (81.5%) patients, while 5 (18.5%)

patients did not show a steroid response. At the end of the induction treatment, remission was achieved in 23 (85.2%) patients. Two patients who were not in remission underwent hematological stem cell transplants. 3 [11.1% (1 in the induction phase, 2 post-induction) patients died from sepsis.

The general characteristics of the patients are summarized in Table 1.

**Table 1.** The general characteristics of the patients

| Patient No | Gender | Age, month | CNS involvement | BCR-ABL | 15.th day MRD (%) | At diagnosis CD49f (%) | 15.th day CD49f (%) | Risk group | Risk groups by MRD | 8.th day MNC/mm$^3$ | 33.th day remission | Exitus | Relapse | HSCT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | M | 27 | - | | 62.0 | 38.4 | 2.3 | MR | HR | 0 | 0 | + (at ind) | - | - |
| **2** | M | 60 | - | | 1.0 | 4.4 | 1.0 | SR | HR | 147 | + | - | - | - |
| **3** | M | 36 | - | | 11.0 | 41 | 11 | MR | HR | 156 | + | - | - | - |
| **4** | F | 114 | - | | 4.0 | 49 | 1.5 | MR | HR | 900 | + | - | - | - |
| **5** | F | 96 | - | | 0.08 | 62.5 | 1.0 | MR | SR | 120 | + | - | - | - |
| **6** | M | 152 | - | | 0.1 | 41 | 6.0 | MR | SR | 0 | + | - | - | - |
| **7** | F | 54 | - | | 6.0 | 11.1 | 7.0 | HR | HR | **4000** | + | - | - | - |
| **8** | M | 48 | - | | 13 | 26 | 1.8 | SR | HR | 0 | + | - | - | - |
| **9** | M | 14 | - | | 4.0 | 52.5 | 1.6 | HR | HR | 278 | + | + (Post-ind) | - | - |
| **10** | M | 21 | - | | 0.1 | 8.0 | 2.0 | SR | SR | 95 | + | - | - | - |
| **11** | F | 168 | - | | 0.02 | 57.7 | 3.8 | MR | SR | 0 | + | - | - | - |
| **12** | F | 132 | - | ] | 40 | 44 | 1.0 | HR | HR | **>1000** | + | - | - | + |
| **13** | F | 94 | + | | 2 | 22.5 | 2.92 | HR | HR | **>1000** | 0 | + (post-ind) | BM | + |
| **14** | M | 17 | - | | 78 | 73 | 66 | SR | HR | 0 | + | - | - | - |
| **15** | M | 32 | - | | 18 | 88.5 | - | HR | HR | **>1000** | + | - | - | - |
| **16** | M | 132 | - | | 0.06 | 30.9 | 1.2 | MR | SR | 0 | + | - | - | - |
| **17** | M | 84 | - | | 22 | 49.8 | 4.5 | HR | HR | 550 | + | - | - | - |
| **18** | F | 60 | - | | 0.16 | 33 | 0.8 | SR | SR | 0 | + | - | - | - |
| **19** | F | 32 | - | | 0.01 | 79.2 | 3.0 | MR | SR | 200 | + | - | - | - |
| **20** | F | 48 | - | | 0.02 | 56 | 6.0 | MR | SR | 0 | + | - | - | - |
| **21** | M | 18 | - | | - | 22 | 1.0 | HR | HR | **2133** | + | - | - | - |
| **22** | F | 60 | - | | 28 | 57 | 1.0 | MR | HR | 0 | + | - | - | - |
| **23** | M | 192 | - | | 0.1 | 5 | 5.0 | MR | SR | 268 | + | - | - | - |
| **24** | M | 192 | - | | 3.0 | 11.8 | 1.4 | MR | HR | 287 | + | - | - | - |
| **25** | M | 183 | - | | 0.01 | 74.5 | 2.0 | MR | SR | 128 | + | - | - | - |
| **26** | F | 22 | - | | 0.02 | 35 | 1.0 | MR | SR | 620 | + | - | - | - |
| **27** | M | 55 | - | | 9.0 | 15 | 7.0 | SR | HR | 400 | + | - | - | - |

CNS: central nervous system, MRD: minimal residuel disease, MNC: mononuclear cell, HSCT: hematopoietic stem cell transplantation, M: male, F: female, Ph: Philadelphia chromosome, SR: strandard risk, MR: medium risk, HR: high risk, ind: induction, BM: bone marrow

### 3.2. MRD analysis

Fifteen (55.6%) patients were MRD$^+$. Among the MRD+ patients, five (33.3%) patients were female. No relationship was found between gender and the MRD$^+$ (p=0.68). The median age in MRD$^+$ patients was 5.6 (1.1-16) years.

Risk groups of MRD-positive patients were 4 (26.7%) SR, 5 (33.3%) IR and 6 (40%) HR respectively. Except for 1 patient, MRD could not be conducted due to lack of sample. All patients in the HR group were MRD positive. 5 (35.7%) IR, and 4 (66.6%) SR patients were classified as HR according to MRD assessment. Furthermore, 9 (64.2%) IR patients were assessed as SR according to MRD. A significant relationship was found between risk groups and the MRD positivity (p=0.02).

In the peripheral smear evaluation on the 8th day, 11 (73.3%) of MRD$^+$ patients had cytomorphological remission. All MRD$^-$ patients had cytomorphological remission on the 8$^{th}$-day evaluation. No correlation was found between MRD positivity and 8$^{th}$-day cytomorphological remission (p=0.11).

On the 15$^{th}$ day, 21 (77.7%) patients had cytomorphological remission. 10 (66.6%) of the MRD$^+$ patients and all the MRD$^-$ patients had cytomorphological remission. No correlation was found between MRD positivity and 15$^{th}$-day cytomorphological remission (p=0.053).

Cytomorphological remission was achieved in 25 (92.5%) patients who achieved remission on the 33$^{rd}$-day bone marrow evaluation. 14 (56%) of these patients had MRD$^+$.

### 3.3. CD49f analysis

CD49f levels at diagnosis and on the 15$^{th}$-day were mean 38.4 ± 22.1 and 5.4±12.6, respectively. There was a statistically significant difference in leukemic blasts' expression of CD49f (p=0.00). In groups MRD$^+$ and MRD$^-$, CD49f levels at diagnosis were mean 38.9±24.0 and 43.8±24.5, respectively (p=0.57). In groups MRD$^+$ and MRD$^-$, 15$^{th}$ day CD49f levels were mean 7.8±17 and 2.8±2 respectively (p=0.64). This expression is not significant for minimal residual disease detection compared to day 15 MRD. No relationship was detected between MRD and CD49f on day 15 (p=0.54).

Therefore, no reclassification was performed according to CD49f. Fig. 3 shows the association between CD49f and MRD on day 15.

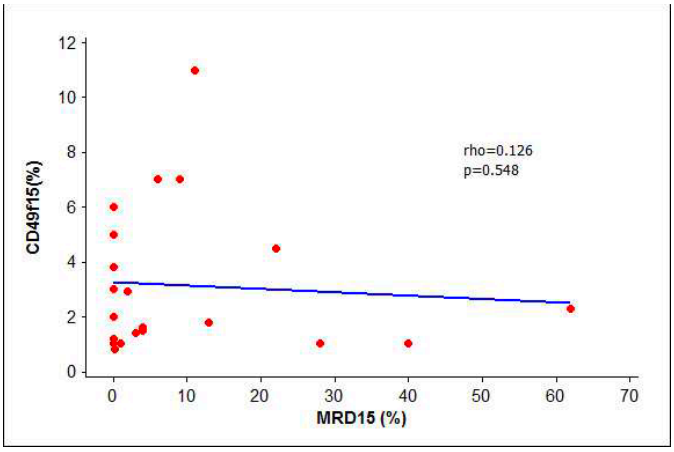


**Fig. 3.**15th day MRD and CD49f

## 4. Discussion

This study aimed to assess the performance and relevance of CD49f as a marker for the detection of MRD. Findings revealed that blasts initially exhibited high levels of CD49f, which then gradually decreased and persisted into the 15th day. However, no correlation was found between MRD and CD49f on day 15.

New approaches in risk classification for leukemia treatment have been developed. However, there was a need for more accurate and less leukemic blast identification techniques for identifying leukemic blasts. Detection of MRD has led to significant improvements in risk stratification and management of ALL (11, 12). Risk-targeted treatment strategies and improved overall survival have been aimed at using MRD detection, clinical, and cytomorphological features together (13, 14).

Numerous markers have been examined for MRD accuracy and usefulness during the last 2 decades. Macedo et al (1995) reported that CD34+ cells do not express CD3, CD20, CD22, CD14, CD65, and CD56, and their combination with CD34+ can be used in MRD examination (15). In another study, an MRD examination was performed with 30 different markers by the FCM method. This study determined that 22 different markers were expressed at different rates in leukemic cells and the relationship between some indicators and genetic abnormalities (8).

Disease recurrence is the primary factor influencing survival rates. Relapse was more likely in SR and IR patients when risk was classified based on cytomorphological characteristics (16). Therefore, MRD detection is very significant in patients in the low-risk group. Many studies have shown high relapse rates in $MRD^+$ (17). These recent data have provided a reclassification of SR patients with $MRD^+$. In our study, $MRD^+$ was in all the HR groups, but there was also a significant $MRD^+$ in the SR and IR groups. Furthermore, all $MRD^-$ and the majority of $MRD^+$ patients had cytomorphologic remission. Due to our short follow-up time, we were unable to assess the relapse rate.

CD49f is also an investigated indicator for MRD (9, 18). DiGiuseppe et al. (2009) evaluated the expression of CD49f in normal B cell maturation and preB-ALL cells. In this study, low CD49f expression was detected in all stages of B cell maturation, as well as moderate CD49f expression in leukemic blasts at the time of diagnosis. CD49f expression had similar results with other antibodies used in MRD. In this study was observed that CD49f could be overexpressed during the induction period (9). In conclusion, this article highlighted that even if CD49f is not detected at diagnosis, it can still be a useful indicator for MRD in follow-up (9). In our study, CD49f expression was high at the diagnosis and significantly decreased by the time of follow-up. We did not observe similar results between CD49f and MRD. However, we found a correlation between a decrease in leukemic blasts and a reduction in CD49f expression.

A recent study reported that 22 markers, including CD49f, are expressed at different rates in normal B-cell and leukemic blasts (8). However, studies on CD49f are not sufficient. Our study is one of the few on CD49f and MRD. In our report, we detected high CD49f expression in leukemic blasts at the diagnosis. Also, we observed that CD49f was expressed higher in the $MRD^+$ patients on day 15 than in the $MRD^-$ patients. We hypothesized that normal B cell expression could be correlated with low CD49f expression in $MRD^-$.

Recently, Collins et al (2021) reported an association between CD49f expression and genetic subgroups of ALL. In this study, significant differences in CD49f expression were detected in 5 genetic subgroups. Particularly in KMT2A-rearranged cases, decreased CD49f expression was revealed (19). Because there was only one patient in our study group with a genetic mutation, we were unable to assess the association between CD49f expression and the genetic subgroup.

ALL has a marked tendency to metastasize to the central nervous system. In a recent study, CD49f-laminin interactions were correlated to the CNS involvement (20). Yao et al (2018) emphasized that CD49f expression enables leukemic blasts to use neural migration pathways (20). In our research, one patient had CNS involvement. This patient had high CD49f expression, and this expression decreased on the $15^{th}$ day. This suggests that CD49f expressed in leukemic stem cells may be resistant to treatment, an increased likelihood of CNS metastases, and a potential? association with the ETV6-RUNX genetic group.

The most significant limitations of our study are the short follow-up time and the small number of patients. The impact of CD49f expression on relapse was not evaluated because of the short follow-up time.

In conclusion, this study demonstrated that CD49f was

significantly expressed in leukemic blasts but was also weakly expressed on the 15th day. We conclude that this expression alone is insufficient to define MRD. However, research with more participants, longer follow-ups, and sequential MRD and CD49f monitoring are required to assess the performance and relevance of CD49f as a marker in the detection of MRD.

**Conflict of interest**

The authors declared no conflict of interest.

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**Authors' contributions**

Concept: T.K., T.P Design: T.P., Data Collection or Processing: T.K, E.Y., Analysis or Interpretation: E.U., M.K., Literature Search: T.K., Writing: T.K.

**Ethical Statement**

Ethical permission required for the study was obtained by Ethic Committee from Erciyes University, with the decision numbers 2011/347.

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