

# Evaluation of CD30 expression in B ALL and its correlation with MRD (Minimum Residual Disease)

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**Background:** This study was conducted to evaluate CD30 expression in minimum residual disease after chemotherapy in B-acute lymphoblastic leukemia (B-ALL). **Materials and Methods:** This was a cross-sectional study on 30 new cases of B-ALL between 2018 and 2019. We checked CD30 expressions in fresh bone marrow aspirates by flow cytometry. After 28 days of routine chemotherapy, we calculated minimal residual disease in CD30 positive and negative patients and compare them by Kolmogorov–Smirnov test. **Results:** Thirty patients with B-ALL with a mean age of  $15.62 \pm 20.488$  were included in the study. CD30 marker was positive in about 10 patients and was negative in about 20 participants. Mean blast count in baseline in CD30 positive group was  $77 \pm 7.88\%$ , in negative group was  $76.3 \pm 17.78\%$  ( $P = 0.292$ ). After 28 days of chemotherapy mean minimal residual disease (MRD) was  $1.07 \pm 3.754$  in the negative group,  $0.12 \pm 0.034$  in the positive group ( $P = 0.025$ ). **Conclusion:** Lower MRD on day 28 after chemotherapy was seen in B-ALL patients with baseline CD30 expression.

**Keywords:** Acute lymphoblastic leukemia, B lymphoblastic leukemia, CD30, flow cytometry, minimum residual disease

**How to cite this article:** Kazemian A, Nematollahi P. Evaluation of CD30 expression in B ALL and its correlation with MRD (Minimum Residual Disease). J Res Med Sci 2021;26:90.

## INTRODUCTION

CD30, known as K1-antigen, is a member of tumor necrosis factor  $\alpha$ -receptors.<sup>[1]</sup> It is expressed in some hematologic malignancies including B-cell and T-cell leukemia.<sup>[2]</sup> Due to its estimated role in the pathogenesis of refractory cases of acute lymphoblastic leukemia (ALL), several therapies such as brentuximab vedotin are now targeted this receptor.<sup>[3]</sup> In ALL patients, the remaining blastic leukemic cells in the bone marrow after chemotherapy regimens which are known as a minimal residual disease (MRD) are defined as a marker of treatment response and are probably an indicator of following relapse.<sup>[4]</sup>

Some studies hypothesized that CD30 expression in primary bone marrow aspiration (BMA) samples might have some relation with the after chemotherapy MRD cells so it could be an indicator of the ongoing remission

and relapse.<sup>[5]</sup> We designed this study to declare whether CD30 positivity at the baseline has any relation with posttreatment MRD or not.

## MATERIALS AND METHODS

This is a cross-sectional study which had been done on fresh BMA samples of 30 definitely diagnosed B-ALL new patients in Isfahan medical university (IUMS) from April 2018 to March 2019. Convenience time-based sequential sampling method was used as our sampling method. We exclude patients whom could not be examined for MRD (due to misplaced marker expression); Participants without BMA samples for flow cytometry and patients with diluted samples. This study is approved by the medical ethics committee of IUMS (IR. MUI. MED. REC.1398.045). BMA was collected after assigning informed consent by patients of his/her parents.

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[10.4103/jrms.JRMS\\_1024\\_20](https://doi.org/10.4103/jrms.JRMS_1024_20)

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**Submitted:** 28-Aug-2020; **Revised:** 17-Mar-2021; **Accepted:** 14-Apr-2021; **Published:** 18-Oct-2021

All BMA samples from the posterior superior iliac spine (PSIS) were obtained by hematologist through Jamshidi cannula (15G mm × 79 mm). BMA samples were collected in EDTA impregnated tubes. The samples were adjusted to approximately 500,000 cells/100 µL with Phosphate-buffered Saline (PBS). Samples were added to 4-color flow cytometry panel of CD30 marker; APC anti-human CD30 antibody, from Biolegend Co.

The samples were analyzed after being centrifuged twice and PBS washing by CyFlow software and a minimum of 30,000 events were acquired and analyzed. The cutoff point for CD30 positivity was 20%. After 28 days of routine anti-ALL therapy<sup>[6]</sup> another BMA was obtained again from PSIS and MRD was calculated, with blastic markers expression, flow cytometry analyzer.

The collected data were entered into SPSS version 25 (SPSS Corp., Chicago, IL, USA).  $P < 0.05$  was considered as the minimum value for statistical significance. Finally, we compare MRD between CD30 positive and negative samples by one-sample Kolmogorov–Smirnov test.

## RESULTS

Finally, 30 patients with B-ALL were included, 12 (40%) were females and 18 (60%) were males. The mean age was  $15.62 \pm 20.488$ . Table 1 summarizes demographic findings.

The cases were categorized with flow cytometry findings into early pre-B ALL (14 patients), pre-B ALL (15 patients), and pro-B ALL (1 patient). CD30 expression in early pre-B was  $17.35 \pm 17.989$ , Pre-B was  $15.01 \pm 23.441$ , in Pro-B was 0.6 and totally was  $15.62 \pm 20.488$ . There were no significant differences in CD30 in B-ALL Immunophenotypes ( $P=0.323$ ). Expression of CD 30 in males was  $17.06 \pm 22.431$  and in females was  $13.48 \pm 17.90$  ( $P = 0.465$ ). There were no significant differences between CD30 expression in different age groups ( $P = 0.465$ ). The blood indices were not significantly difference with CD30 expression [Table 2].

CD30 was positive in 20 participants and negative in 10 patients. The mean blast count in baseline in CD30 positive group was  $77 \pm 7.88$ , in the negative group was  $76.3 \pm 17.78\%$ . After 28 days of chemotherapy mean MRD was  $1.07 \pm 3.754$  in the CD30 negative group,  $0.12 \pm 0.034\%$  in the positive group [Figure 1]. Total MRD count was  $0.75 \pm 3.073$  in participants. MRD levels were statistically different two groups ( $P=0.025$ ).

## DISCUSSION

This study was conducted to show any relation between baseline CD30 positivity in B-ALL patients and their posttreatment MRD level.

**Table 1: CD30 marker expression in terms of age, sex, and acute leukemia immunophenotyping of the patients**

Variables	CD30 expression, mean (percentage)±SD	P
Sex		
Male	17.06±22.431	0.465
Female	13.48±17.90	
Total	15.62±20.48	
Age (years old)		
<10	17.30±20.651	0.800
10-20	18.11±29.970	
20-30	12.75±17.890	
30-40	2.00±00.00	
>40	2.80±3.818	
Total	15.62±20.488	
ALL immune phenotyping		
Early preB	17.35±17.989	0.323
Pre-B	15.01±23.441	
Pro-B	0.60±00.00	
Total	15.62±20.488	

ALL=Acute lymphoblastic leukemia; SD=Standard deviation

**Table 2: Levels of blood indices in terms of the CD30 marker being positive or negative**

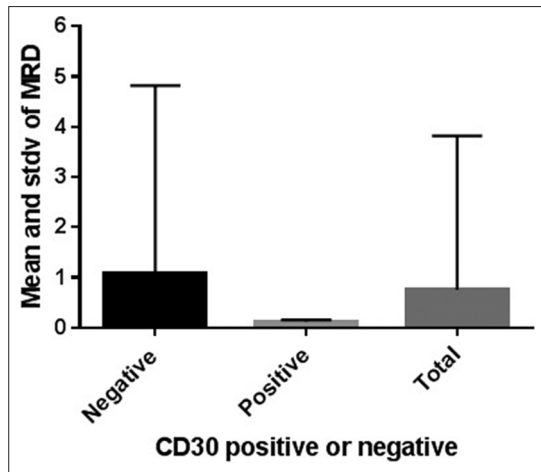
Variables	Mean±SD		P
	CD30 negative	CD30 positive	
WBC	37441.00±50944.10	22982.00±32995.90	0.713
Hb	9.23±2.05	8.19±1.99	0.267
PLT	78350.00±68172.59	80100.00±54726.49	0.619
MCV	85.61±9.47	84.10±8.33	0.779
MCH	27.52±2.69	26.67±2.24	0.397
RDW	17.56±3.19	16.23±1.57	0.198
Blast	76.30±17.789	77.00±7.88	0.307

SD=Standard deviation; WBC=White blood cells; Hb=Hemoglobin; PLT=Platelet; MCV=Mean corpuscular volume; MCH=Mean corpuscular hemoglobin; RDW=Red cell distribution width

Kalinova *et al.*<sup>[7]</sup> concluded that using the CD30 molecule to determine MRD is not a viable method. This finding is not consistent with the results of the present study. Elafifi *et al.* showed that an increase in MRD was not statistically significant in ALL relapsing cases compared to new cases. Accordingly using the CD30 molecule to identify and diagnose MRD was not opted to be a viable option.<sup>[8]</sup> Levels of MRD have a prognostic value in many hematologic malignancies.<sup>[9-15]</sup>

A meta-analysis study also found that high serum CD30 levels were a predictor factor of poor prognosis in patients with Hodgkin's lymphoma which is quite different from our study.<sup>[16]</sup> The serum CD30 level decreases with the treatment of the disease and returns to its highest levels in case of relapse.<sup>[17]</sup> As we showed CD30 positive patients had lower MRD, it might be a good prognostic factor and a positive indicator of treatment response.

Limitation of this study was the limited sample size and short clinical follow-up time which were due to budget



**Figure 1:** Number of blasts on day 28 after starting the treatment (minimal residual disease) by a multicolored flow cytometry method

constraints, resulting in a need for further studies with higher sample size and extended follow-up to obtain more valid and established evidences.

## CONCLUSION

Lower MRD on day 28 after chemotherapy was seen in B-ALL patients with baseline CD30 expression.

## Financial support and sponsorship

This study was supported by Isfahan University of Medical Sciences.

## Conflicts of interest

There are no conflicts of interest.

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