Research Article

Role of CD58 in minimal residual disease detection of ALL patients at South Egypt Cancer Institute

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Abstract

Objective: To determine the role of CD58 marker in Minimal Residual Disease (MRD) monitoring in B-cell Precursor ALL patients at South Egypt Cancer Institute (SECI). **Methodology:** This study was carried out in Department of Clinical pathology, South Egypt Cancer Institute, from October 2017 to December 2019. We used SECI Flowcytometry lab 4 color acute leukemia panel for diagnosis, then we studied the role of CD58 in detection of MRD by applied it twice; at diagnosis and at day21 (post induction). **Results:** 50 patients were included in our study with acute lymphoblastic leukemia (ALL) who underwent CBC, BM aspiration and immunophenotyping. The mean age was 9.14±5.53 years. It included 31 (62%) males and 19 (38%) females. MRD by CD58 was positive in 18 (36%) of patients while was negative in 32 (64%). CD58 show sensitivity of 100%, specificity of 97%, positive predictive value of 94.4% and negative predictive value of 100% whereas the overall diagnostic accuracy of CD58 in MRD detection in ALL patients was calculated to be 98%. **Conclusion:** CD58 showed a high sensitivity to assess MRD in ALL patients, so its use to detect MRD in ALL is very useful.

Keywords: ALL, CD58, MRD

Introduction

Complete remission (CR) was defined as <5% leukemic blasts in the bone marrow sample reviewed at the time of peripheral blood count recovery, absence of circulating peripheral blasts, absence of extra medullary disease, platelet count ≥100 × 109/L, and absolute neutrophil count ≥ 1.0 × 109/L. MRD relapse was defined as recurrence of detectable MRD (sensitivity for positive value 0.01%), despite persistence of clinical complete morphological remission. Morphological relapse was defined as presence of leukemic blasts in any extra medullary location, or in the bone marrow or peripheral blood at a level of >5% (Pemmaraju N, et al.,, 2017).

National Cancer Institute has incorporated MRD of > 0.01% in post induction marrow as high risk (HR) feature. Most studies use cutoff value of >0.01% in BM mononuclear cells (MNCs) as MRD HR and < 0.01% as MRD low risk (Parikh and Uparkar, 2016).

However, the morphological analysis is complicated by the fact that ALL blast cells often highly resemble the appearance of normal lymphoid precursors (hematogones). Minimal residual disease (MRD) testing employs the use of highly sensitive assays, most commonly by flow cytometry, to identify residual leukemic cells that may be undetected by morphological assessment alone (Setiad et al.,, 2016).

CD58 has been proposed as playing a role in flowcytometry MRD and is currently used in the COG protocol (Tsitsikov et al.,, 2018).

Methodology

This cross-sectional study was conducted on 50 patients newly diagnosed with

Precursor B-ALL who were admitted to the SECI after approval by hospital ethical committee and taking consent, during the period between October 2017 to December 2019. Patients consists of 31 males and 19 females, with an age ranged from 2 to 26 years old.

In our study we exclude patients who were previously diagnosed or relapsed ALL.
In our experience **Complete blood count (CBC)** was performed by CELL‑DYN 3500 (Abbott Diagnostics, Santa Clara, California, USA) twice; at diagnosis and post induction therapy at day 21. Also bone marrow aspirate was done for patients twice; at time of diagnosis, and at day 21 post induction for tracing of MRD.

Routine immunophenotyping was performed with a panel of fluorochromes including: Flurescence isothiocyanate (FITC), Phycoerythrin (PE), Peridinin Chlorophyll Protein Complex (PerCP), Allophycocyanin (APC), Conjugated monoclonal antibodies.

The used tubes in monitoring and assay of MRD in precursor B-ALL.was as the following:
1- MRD tube I: CD58 FITC/CD10 PE/ CD34 Percp/CD19 APC (BD BioScience).
2- MRD tube II: CD38 FITC/CD10 PE/ CD34 Percp/CD19 APC (BD BioScience).
Analysis was done by multicolor flow cytometry (FACS Calibur, (BD) Biosciences-San Jose, CA, USA, serial number E5140) (2 lasers, 4 color flow cytometry) using the Cell-Quest Pro software program (Becton Dickinson).

Gating was done by experienced professor. The cut off for MRD was 0.01%.
Data Analysis: The data was analyzed using SPSS 20. Categorical variables like gender and true positives were presented as frequencies and percentage. For numerical variables like age, mean standard deviations were presented.

Results

We studied 50 patients with the age from 2 to 26 years. The mean age was 9.14±5.53 years. It included 31(62%) males and 19 (38%) females (figures 1 and 2).

Out of the Fifty ALL patients at diagnosis, 43/50 (86%) patients had anemic manifestations, 41/50 (82%) patients had Fever, 40/50(80%) patients had spleno-megaly, 28/50 (56%) patients had bone tenderness, 26/50(52%) patients had hepatomegaly, 19/50 (38%) patients had lymphadenopathy, 15/50 (30%) patients had bleeding, 13(26%) patients had purpura and 9/50 (18%) patients had recurrent infection (figure 3).

Hematological features (white blood cells count, platelet count and Hb level) for the studied patients both at diagnosis and post induction are shown in table (1).
Immunophenotyping of the studied patients by using FACS Calibur flow cytometry at diagnosis data shown in figure (4).

We reveled that there was high significant difference in CD58 percentage expressions both at diagnosis and post induction (P= < 0.001). While there was no significant statistical difference of CD58 MFI at diagnosis and post induction (P= 0.460) data shown in table (2).

Our study showed that the percentage of MRD cells detected by CD58 ranged from 0 to 1.23%, with a mean value of 0.069±0.205 % and MRD by CD58 was positive in 18 (36%) of patients while was negative in 32 (64%). (Table 3).

In our study; CD58 show sensitivity of 100%, specificity of 97%, positive predictive value of 94.4% and negative predictive value of 100% whereas the overall diagnostic accuracy of CD58 in MRD detection in ALL patients was calculated to be 98% (Table 4)

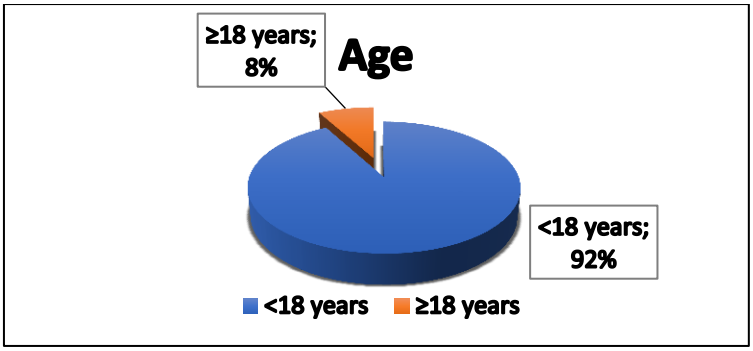

Figure (1): Age distribution of the studied precursor B-ALL patients

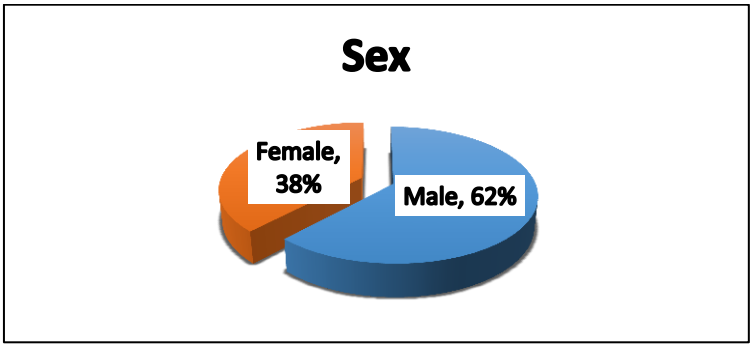

Figure (2): Sex distribution of the studied precursor B-ALL patients

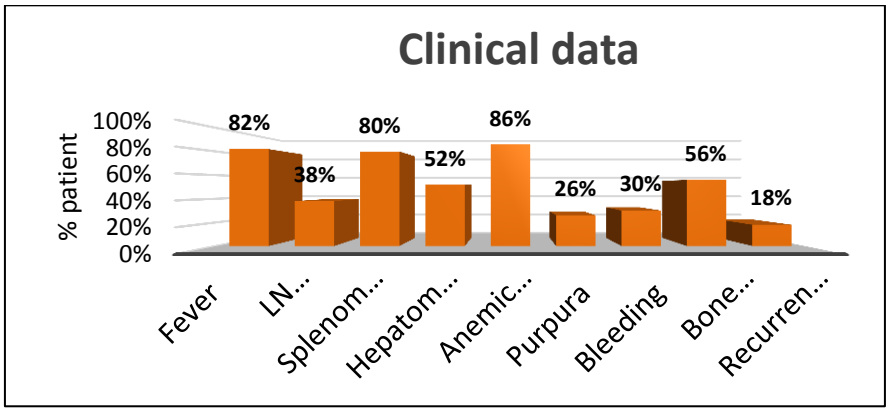

Figure (3): Clinical data of the studied precursor B-ALL patients at diagnosis

Table (1) Association between Laboratory data at diagnosis and post induction of the studied precursor B-ALL patients:

	At diagnosis	*Post-induction*	*Test*	*p value*
WBCs (x10^9/L) Mean±SD Median (Range)	 31.13±44.41 7.9 (1.4-169.9)	 4.26±3.22 3.55 (1.1-15.8)	-4.422	**<0.001***
Hb (g/dL) Mean±SD Median (Range)	 9.86±2.13 9.95 (6.3-16.8)	 9.75±1.78 9.75 (1.2-13.4)	-0.114	0.909
Platelet (x10^9/L) Mean±SD Median (Range)	 72.48±69.46 51.5 (10-343)	 178.12±112.38 145.5 (41-557)	-5.952	**<0.001***

Wilcoxon Signed Ranks test

: Significant level at P value < 0.05

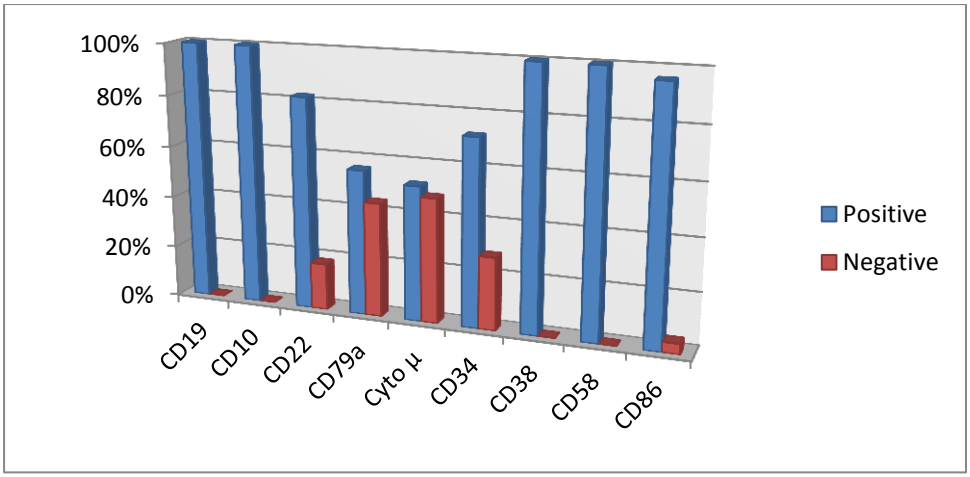

Figure (4): Immunophenotypic markers of the studied precursor B-ALL patients at diagnosis

Table (2): Association between CD58 expression percentage and MFI at diagnosis versus post-induction (n=50).

	At diagnosis	Post-induction	Test	p value
CD58 % Mean±SD Median (Range)	79.24±19.04 85.7 (15.85-99.02)	0.07±0.2 0.01 (0-1.23)	-6.154	**<0.001***
CD58 MFI (N=18) Mean±SD Median (Range)	66.72±27.66 59.1 (37.17-146)	72.06±53.8 47.9 (14.95-186.06)	-0.103	0.918

Mann-Whitney test
† **Wilcoxon Signed Ranks test**
: Significant level at P value < 0.05

Table (3): MRD detected by CD58:

MRD	By CD58
Mean±SD	0.069±0.205
Median(Range)	0.01 (0-1.23)
Positive(>0.01%)	18 (36.0%)
Negative	32 (64.0%)

Table (4): Sensitivity of CD58 compared to CD38 and CD58:

C58 MRD	MRD (CD38+CD58)	
	Positive (n=17)	Negative (n=33)
Positive (n=18)	17 (100%)	1 (3.03%)
Negative (n=32)	0 (0%)	32 (96.97%)
Sensitivity: 100% **Specificity: 97%** **PPV: 94.4%** **NPP: 100%** **Accuracy: 98%**		

Discussion

In the present study, the age of ALL patients ranged between 2 and 26 years, with a mean ± SD of 9.14±5.53 years and a median of 9.5 years; They were 46 (92%) children and 4(8%) adults, being more frequent in children. This was in accordance with Ahmed and Hassab (2008), Siegel et al., (2017) and Mona et al., (2018) who proved the frequency of ALL in children more than adults.

Regarding sex, 31(62%) patients were male and 19(38%) were female with male to female ratio 1.6:1. This was in accordance with previous studies by Willman et al., (2005) and Siegel et al., (2017) who confirmed a male predominance in ALL patients.

Among the patients; 86% had anemic manifestations, 82% had Fever, 80% had splenomegaly and 52% had hepatomegaly, this disagrees with El-Sharkawy et al., (2003), who found splenomegaly in 60% of patients and hepatomegaly in 83.9% of patients.

The Egyptian study done by Ahmed and Hassab (2008) detected lymphadenopathy in 73.3% of their patients. This is more than the present study detecting lymphadenopathy being 38% in our patients but higher than that detected by Pui et al., (2010) who detected only 10% of patients with lymphadenopathy.

Shaver (2014) has illustrated the role of CD58 in MRD detection in ALL patients as CD58 is usually expressed brightly and frequently over-expressed in B-ALL making it a marker similar to CD10 in that, even when not definitively over-expressed, still is present as a dyssynchronous immature marker in combination with other markers of maturity. This was confirmed in our study.

In our study, we observed that CD58 was overexpressed. This agreed with Ramalingam et al., (2020) who observed that many MRD positive cases showed loss of diagnostic LAIP (bright CD10, over-expressed CD58) at MRD assessment.

Our study also was in agreement with Xu et al., (2006) who demonstrated that, CD58 could be an effective marker in MRD detection in B-ALL patients, which would enrich the combination of MRD detection. As well, Shaver et al., (2015) developed an optimized single, eight-color tube for the detection of MRD and noted that CD38 × CD58 was one of the most useful pairs they evaluated. While on the other hand, Theunissen et al., (2017) found that CD58, and CD22 appeared be of limited value in their study. Also, Our results were not in agreement with Baraka et al., (2017) as their study showed that, CD58 was informative in 59.5% only and over expression of CD58 (on CD34 +ve blasts) was recorded in 37.3% of their studied patients.

Conclusion

Minimal residual disease assessment has a very useful role in treating ALL patients. So choosing a very sensitive marker as CD58 will help greatly in MRD detection in ALL patients.

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