Evaluation of CD66c expression for minimal residual disease in precursor B acute lymphoblastic leukemia

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Abstract:

Background: Acute lymphoblastic leukemia (ALL) is a malignant clonal expansion of lymphoid hematopoietic precursors that exhibit developmental arrest at varying stages of differentiation. Two subtypes are defined, according to which lymphoid progenitor is affected: B-cell-precursor ALL (B-ALL) and T-cell ALL (T-ALL). The incidence of ALL differs with age; there is an early peak at 4 to 5 years, a decline in young adults, followed by a slight increase after 50 years of age. Minimal residual disease (MRD) is the name attributed to the very low number of blast cells remaining in the patient during or after treatment (in the remission period). MRD detected in early phases of therapy is shown to provide prognostic information. MRD has proven to be the strongest prognostic factor. MRD-based treatment strategies further improve outcome in the involved patients.

Aim of the study: 1) To evaluate CD66c as a marker for minimal residual disease by flow cytometry in B-ALL patients. 2) To assess the effect of CD66c on the treatment outcomes and overall survival of patients with B-ALL.

Methodology: This is a prospective study which was conducted at Clinical Pathology department, South Egypt Cancer Institute, Assiut University in the period between January 2019 and December 2020. Patients included in the study underwent the standard clinical examination and laboratory evaluation followed by bone marrow aspiration. Sixty B-ALL patients (n=60) underwent flowcytometric analysis for CD66c. Out of the sixty patients (n=60) included at the first time, forty patients (n=40) were re-evaluated at the post-induction phase.

Results: This study included 60 patients, 36 (60%) males and 24 (40%) females. The patients' ages ranged between 2 and 25 years with median age of 6 years. CD66 was expressed in 70% of our B-ALL patients. There was statistically significant difference between the level of expression of CD66c both before and after treatment (p=0.000). No significant correlations were found between level of expression of CD66c and WBCs, PB blasts or BM blast cells. There was no significant correlation found between CD66c and OS. Bone marrow aspirate samples analysed on the post-induction phase showed that CD66c was still stably expressed.

Conclusion: CD66c is highly expressed in our B-ALL patients and is stably expressed after induction of treatment, so its addition in MRD panels can contribute to increasing the sensitivity of the assay. As regard its prognostic value, we couldn't precise its use for evaluation of overall survival in B-ALL patients.

Keywords: acute lymphoblastic leukemia, minimal residual disease, CD66c, flowcytometry

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Introduction:

Acute lymphoblastic leukemia (ALL) is a malignant clonal expansion of lymphoid hematopoietic precursors that exhibit developmental arrest at varying stages of differentiation. Two subtypes are defined, according to which lymphoid progenitor is affected: B-cell-precursor

ALL (B-ALL) and T-cell ALL (T-ALL). The incidence of ALL differs with age; there is an early peak at 4 to 5 years, a decline in young adults, followed by a slight increase after 50 years of age [1].

Minimal residual disease (MRD) is the name attributed to the very low number of blast cells remaining in the patient during or after treatment (in the remission period). MRD detected in early phases of therapy is shown to provide prognostic information [2], where chemotherapy-resistant blast cells in bone marrow (BM)- surviving in small amounts- have capacity to trigger future relapses [4].

New pieces of information are obtained through immunophenotyping, cytogenetics and genomic profiling. Chemotherapy resistance have contributed to a better understanding of the pathology of this complex disorder and to recognition of subgroups of patients who respond differently to therapy [5].

Although most adults with ALL enter complete remission (CR), only 30% to 40% survive 5 or more years, at which time they are considered cured. Survival depends on risk factors such as age, white blood cell count, disease immunophenotype, cytogenetics, and molecular abnormalities. However, these risk factors often lack prognostic precision. In fact, a considerable proportion of standard-risk (SR) patients treated with standard chemotherapy will eventually relapse-up to 40% to 50%. Paradoxically, approximately 20% to 25% of high-risk (HR) patients do not relapse. Therefore, the most important challenge is to establish a more precise prognostic definition to make better therapeutic decisions [7]. Several studies of childhood and adult ALL identified MRD as an important independent prognostic factor [8-10]

There was a progress in the treatment of childhood ALL over the last four decades with cure rates (i.e. no evidence of disease for 10 years or more) now exceeding 90% [11].

Aim of the study:

The aim of this work was:

1) To evaluate new marker (CD66c) for minimal residual disease by flow cytometry in B-ALL patients.

2) To assess the effect of CD66c in relation to CD34 on the treatment outcomes and overall survival of patients with B-ALL.

Patients and Methods:

This is a prospective study that was carried out at the Clinical Pathology Department, South Egypt Cancer Institute (SECI), Assiut University during the period between January 2019 and December 2020. The study was carried on 60 newly diagnosed B-ALL patients, recruited from Pediatric, Medical Oncology Departments and outpatient clinics, SECI, Assiut University.

Evaluation of our studied participants at the first presentation:

Sixty patients (n=60) were included in the study and underwent the standard clinical examination and laboratory evaluation including:

- Full History Taking: with special emphasis on CNS manifestation, testicular swelling in boys and previous treatment with steroid.
- Physical Examination: with special emphasis on lymph nodes and presence of organomegaly.
- Laboratory Investigations:
- Complete blood count.
- Hepatic, renal function tests and uric acid.
- Electrolyte panel (Na, K, Mg, Ca).
- BM aspirate with morphologic examination and immunohistochemistry using Periodic Acid Schiff (PAS) and myeloperoxidase (MPO).
- Immunophenotyping for B and T-ALL.
- MRD detection by flow cytometry for B-ALL patients.
- CSF cytology.
- Radiological studies: Chest X-ray and abdominal ultrasonography.
- Flowcytometric analysis of CD66c.

Evaluation of patients in the follow up:

Out of the sixty patients (n=60) included at the first presentation, forty patients (n=40) were completed the induction remission phase and re-evaluated at the post-induction phases. The rest of the patients were missed early during induction due to death, referral to other hospitals or escaping treatment.

- Clinical, laboratory and flowcytometric evaluation of patients:
- All the patients included in the follow-up underwent the standard clinical examination and laboratory evaluation as previously discussed. Also, they underwent flowcytometrical analysis of CD66c.

Detection of relapse:

Patients who achieved remission after induction phase were passed to complete their treatment (consolidation then continuation phases) and followed up for the occurrence of relapse. We classified BM response according to [12]:

- M3 bone marrow (relapse): patients with ≥25% lymphoblasts among nucleated cells in the BM.
- M2 bone marrow: the presence of leukemic lymphoblasts at levels lower than 25% in BM after complete remission required further study done for diagnosis of relapse.
- M1 bone marrow (CR): patients with no lymphadenopathy or organomegaly detected. CBC returned to normal ranges, disappearance of blast cells from PB, the BM blast cells <5%, normal marrow cellularity and presence of all cell lines.

Survival analysis:

We followed-up our participants for 24 months. Overall survival (OS) was calculated for each patient from the date of diagnosis until patient's death from any cause or date of last follow-up.

Survival rates were estimated at two time-points for each marker: (at 10 and 20 months for CD66c and CD34). These time-points are statistical points.

Statistical Analysis:

All statistical calculations were done using SPSS (statistical package for the social science; SPSS Inc., Chicago, IL, USA) version 26.

Data was statistically described in terms of mean ± standard deviation (±SD), or median and range when not normally distributed, frequencies (number of cases) and relative frequencies (percentages) when appropriate.

Comparisons of quantitative variables were done using Mann Whitney U test because the data was not normally distributed. Comparisons of paired quantitative variables were done by Paired sample t test for normally distributed data or Wilcoxon signed rank test for non- normally distributed data. For comparing categorical data, Exact test was used instead of Chi square ($\chi 2$) test because the expected frequency is less than 5. McNemar test was used for comparing paired binomial data. Correlations between various variables were done using Pearson correlation or Spearman rho test.

Kaplan-Meier test was used to compare survival between both groups (low and high expression). P-value is always 2 tailed set significant at 0.05 level.

Results:

The results are presented under the following sections:

- Section (1): Characteristics and baseline laboratory results of all studied participants (n=60) (Tables 1&2)
- Section (2): Comparison of laboratory data and mean level of expression of CD66c and CD34 before and after induction of treatment (n=40). (Tables 3-7) & (Figures 1-3).
- Section (3): Survival analysis of studied participants (n=40) (Tables 8-10) & (Figures 4&5).

Section 1: Characteristics and baseline laboratory results of all studied participants (n=60) (Tables 1&2)

Table 1 shows that our studied participants were 53 (88.3 %) children and 7 (11.7 %) adults with mean age (11.08 ± 10.73) years and ranged from 2 years up to 25 years (median 6 years). Male patients were 36 (60%) while females were 24 (40%). All of our studied participants were diagnosed as B-ALL, n=60 (100%) based on morphological and immunophenotypic results.

Table 2 shows the baseline laboratory results of all patients:

- The mean hemoglobin value of our patients was 9.38 ± 1.73 g/dl and ranged from 5.4 to 12.9 g/dl.
- The mean platelet count was 68.68 ± 78.19 x 103/µl and ranged from 9 to 405 x 103/µl.
- The mean white blood cells (WBCs) count was 24.70 ± 36.97 x 103/µl and ranged from 1.6 to 168.7 x 103/µl.
- The mean value of peripheral blasts (PB) blast cell count was 49.63 % ± 26.28 and ranged from 3 % to 97%.
- Finally, the mean value of BM blast cell count was 75.58 % ± 20.49 and ranged from 22% to 99%.

Table (1): Characteristics of all studied participants

Variable		n=60	
		N	(%)
Age (years), Children (<18)		53	88.3
Age (years), Adults (≥18)		7	11.7
Age (years), Mean ± SD		11.08 ± 10.73	
Age (years), Median (range)		6 (2 – 25)	
Sex	Male	36	(60.0)
	Female	24	(40.0)
Diagnosis	B-ALL	60	(100)

Quantitative data are presented as mean ± SD and median (range), qualitative data are presented as n (%). SD: standard deviation.

Table (2): Baseline laboratory results of all studied participants

Variable		N=60
Hemoglobin g/dl	Mean ± SD	9.38 ± 1.73
	Median (range)	9.65 (5.4–12.9)
Platelets x 10^3/µl	Mean ± SD	68.68 ± 78.19
	Median (range)	44.50 (9.0–405.0)
WBCs x 10^3/µl	Mean ± SD	24.70 ± 36.97
	Median (range)	13.05 (1.6–168.7)
PB blast cells %	Mean ± SD	49.63 ± 26.28
	Median (range)	44.0 (3.0–97.0)
BM blast cells %	Mean ± SD	75.58 ± 20.49
	Median (range)	81.5 (22.0–99.0)

Quantitative data are presented as mean ± SD and median (range). SD: standard deviation, PB: peripheral blood, BM: bone marrow.

Section 2: Comparison of laboratory data and mean level of expression of CD66c and CD34 before and after induction of treatment (n=40). (Tables 3-7) & (Figures 1-3).

Among 60 patients who were assessed at diagnosis, there were 40 patients that were assessed at the end of induction phase, the other 20 patients were missed during the study due to death, referral to other hospitals or escaping treatment:

Table 3 shows that the mean hemoglobin value of our patients was 9.5 ± 1.59 g/dl and ranged from 5.4 g/dl to 12.9 g/dl, while after treatment it was 10.65 ± 1.24 g/dl and ranged from 8.8 g/dl to 12.6 g/dl.

The mean platelet count was 73.75 ± 91.87 x 103/µl and ranged from 10 x 103/µl to 405 x 103/µl, while after treatment it was 204.50 ± 167.36 x 103/µl and ranged from 15 x 103/µl to 604 x 103/µl.

The mean WBCs count was 23.27 ± 35.87 x 103/µl and ranged from 1.6 x 103/µl to 166 x 103/µl, while after treatment it was 5.29 ± 7.22 x 103/µl and ranged from 0.6 x 103/µl to 34.6 x 103/µl.

The mean number of PB blast cell count was 45.98 ± 27.03 % and ranged from 3 %to 97 %, while after treatment it was 0.25 ± 1.58 % and ranged from 0 % to 10 %.

Finally, the mean number of BM blast cell count was 77.63 ± 16.12 % and ranged from 50 % to 98 %, while after treatment it was 3.78 ± 4.83 % and ranged from 1% to 24%.

There were statistically significant differences between values before and after treatment concerning all baseline laboratory data (hemoglobin, platelet count, WBCs count, PB blast cells count and BM blast cells count), P=0.000.

Table (4) shows comparison of number of cases (defined as high and low expression) between before and after treatment:

- Considering CD66c, cases with high expression were considered at cutoff ≥ 20% and low expression below 20% (13), number of cases showing high level of expression was 28 (70%) before treatment, while after treatment there were 12 cases (30%) with high level of expression.
- Considering CD34, a cutoff value of ≥ 10% was considered for high expression (Zhang et al., 2020), number of cases showing high level of expression was 27 (67.5%) before treatment, while after treatment there were 4 cases (10%) with high level of expression.
- There were statistically significant differences between the level of expression of the CD66c and CD34 both before and after treatment, P= 0.000 & 0.000 respectively.

Table (5) shows that:

- Considering CD66c level of expression before treatment, mean value of CD66c was 38.56 ± 32.49 and ranged from 0.3 to 96 while after treatment it was 15.28 ± 20.54 and ranged from 0.2 to 90.8.
- Also, mean value of CD34 before treatment was 38.48 ± 29.65 and ranged from 0.4 to 81.5 while after treatment it was 4.04 ± 5.54 and ranged from 0.08 to 20.
- There were statistically significant differences between the level of expression of the CD66c and CD34 both before and after treatment, P= 0.000 & 0.000 respectively. This is also shown in (Figures 1 & 2) respectively.

Table 6 shows that there were no significant correlations between CD66c and WBCs, PB blasts or BM blast cells.

Table 7 shows that there were significant moderate positive correlations was found between level of expression of CD34 and CD66c (r = 0.488) as shown in (Figure 3).

Table (3): Comparison of laboratory data before and after induction of treatment of B-ALL patients (n=40)

Variable		Before induction Treatment	After induction Treatment	P value
Hemoglobin g/dl	Mean ± SD Median (range)	9.50 ± 1.59 9.69 (5.4–12.9)	10.65 ± 1.24 10.85 (8.8–12.6)	0.000*
Platelets x 10^3/µl	Mean ± SD Median (range)	73.75 ± 91.87 32.0 (10.0–405.0)	204.50 ± 167.36 129.5 (15–604)	0.000*
WBCs x 10^3/µl	Mean ± SD Median (range)	23.27 ± 35.87 11.5 (1.6–166.0)	5.29 ± 7.22 3.2 (0.6–34.6)	0.000*
PB blast cells x 10^3/µl	Mean ± SD Median (range)	45.98 ± 27.03 40.0 (3.0–97.0)	0.25 ± 1.58 0.0 (0.0–10.0)	0.000*
BM blast cells x 10^3/µl	Mean ± SD Median (range)	77.63 ± 16.12 83.0 (50.0–98.0)	3.78 ± 4.83 2.5 (1.0–24.0)	0.000*

Quantitative data are presented as mean ± SD and median (range). Paired sample t test was used for the quantitative variables which are normally distributed (Hemoglobin), Wilcoxon signed rank test for non- normally distributed paired data (Platelets, WBCs, PB blast cells and BM blast cells). Significance was defined by $p < 0.05$. SD: standard deviation, PB: peripheral blood, BM: bone marrow.

Table (4): Comparison of number of cases (defined as high and low expression of studied markers) between before and after induction of treatment

Tumor marker		Before		After		p-value
		N	(%)	N	(%)	
CD66c	< 20%	12	(30.0)	28	(70.0)	0.000*
	≥ 20%	28	(70.0)	12	(30.0)	
CD34	< 10%	13	(32.5)	36	(90.0)	0.000*
	≥ 10%	27	(67.5)	4	(10.0)	

Qualitative data are presented as n (%). McNemar test was used for comparing paired binomial data. Significance defined by $p < 0.05$. CD: cluster of differentiation

Table (5): Comparison of the mean level of expression of CD66c and CD34 before and after induction of treatment (n=40)

Variable		Before Treatment	After Treatment	P value
CD66c	Mean ± SD	38.56 ± 32.49	15.28 ± 20.54	0.000*
	Median (range)	27.6 (0.3–96.0)	8.25 (0.2–90.8)	
CD34	Mean ± SD	38.48 ± 29.65	4.04 ± 5.54	0.000*
	Median (range)	47.8 (0.4–81.5)	2.5 (0.08–20.0)	

Quantitative data are presented as mean ± SD and median (range). Wilcoxon signed rank test was used for comparing paired quantitative variables because they were not normally distributed. Significance defined by $p < 0.05$. SD: standard deviation, CD: cluster of differentiation.

Table (6): Correlation between the studied CD66 and different laboratory data (n=60)

Tumor marker		WBCs	Peripheral blasts	BM blasts
CD66c	r	-0.151	-0.137	0.007
	p-value	0.250	0.297	0.958

*Significance defined by $p < 0.05$, r =correlation coefficient

Table (7): Correlations between CD 66c and CD34 (n=60)

Tumor marker		CD66c
CD34	r	0.488
	p-value	0.000*

*Significance defined by $p < 0.05$, r=correlation coefficient. CD: cluster of differentiation.

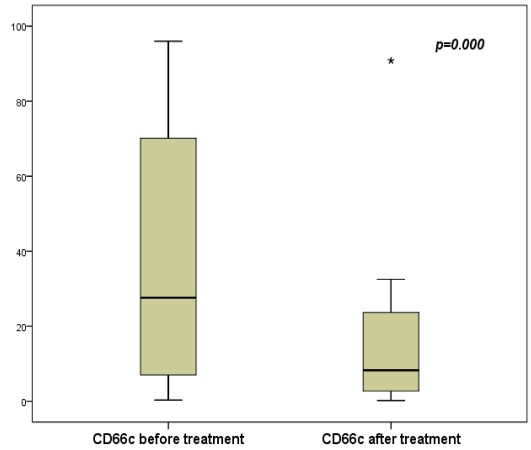

Figure. (1): Box plot graph shows CD66c level of expression in the studied ALL patients before and after induction of treatment

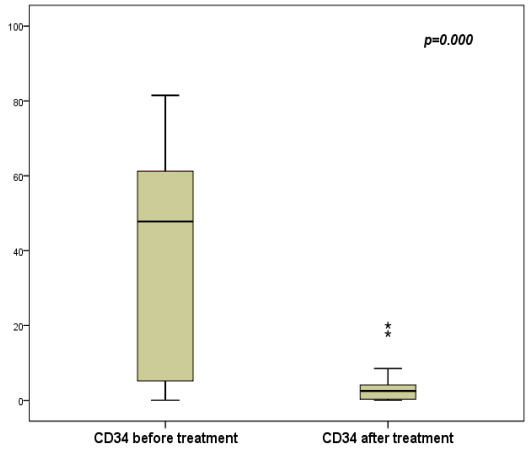

Figure (2): Box plot graph shows CD34 level of expression in the studied ALL patients before and after induction of treatment

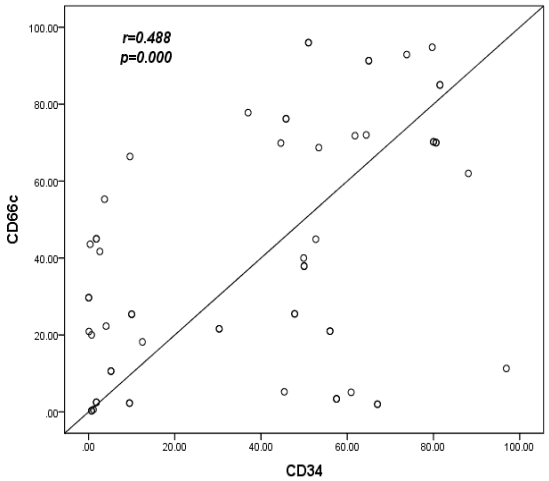

Figure (3): Scatter plot graph showing the correlation between CD34 and CD66c

Section 3: Survival analysis of studied participants (n=40) (Tables 8 - 10) & (Figures 4&5).

The markers are classified according to the magnitude of expression into positive (high expression) and negative (low expression). Accordingly, the survival analysis was done depending on the level of expression of CD66c and the results are listed below:

Table 8 and Figure 4 show that there was no significant difference in the OS of the studied group at both time points (10 months, 20 months) between low and high expression of CD66c (P=0.901).

Table 9 and Figure 5 show that there was a higher incidence of survival in the (low expression) group of CD34 at both time points (10 months, 20 months) where 100% of patients survived, than in the (high expression) group, yet it was not a statistically significant difference in the OS between both groups (P=0.072).

Table 10 shows that the mean value for CD66c in patients who were in complete remission (CR) was 35.01±32.31 while in relapsed patients, it was 47.89±32.62 and however increased value in relapsed patients but the results are not statistically significant. Also, the mean value for CD34 in patients who were in CR was 30.02±25.49 while in relapsed patients, it was 60.77±29.23. There was a statistically significant difference between CD34 mean value in CR and relapsed group of patients (P= 0.000).

Table (8): Overall survival according to CD66c level of expression

OS according to CD66c	Estimate ± SE		P-value
	Low expression	High expression	
At 10 months	83.3±10.8%	85.7±6.6%	0.901
At 20 months	83.3±10.8%	85.7±6.6%	

*The values are expressed as % of patients who survived at the end of the determined duration. Significance defined by p < 0.05. SE: standard error, CD: cluster of differentiation.

Table (9): Overall survival according to CD34 level of expression

OS according to CD34	Estimate ± SE		P-value
	Low expression	High expression	
At 10 months	100±0.0%	77.8±8.0%	0.072
At 20 months	100±0.0%	77.8±8.0%	

*Significance defined by p < 0.05. SE: standard error, CD: cluster of differentiation.
*The values are expressed as % of patients who survived at the end of the determined duration.

Table (10) Comparison of CD66c according to the response status of the studied participants before and after induction of treatment

Variable	Mean value before induction of CTH		p-value
	CR (n=29)	Relapsed (n=11)	
	Mean ± SD	Mean ± SD	
CD66c	35.01±32.31	47.89±32.62	0.455

Quantitative data are presented as Mean ± SD. Mann Whitney U test was used for comparing quantitative variables because they were not normally distributed. Significance defined by p < 0.05.

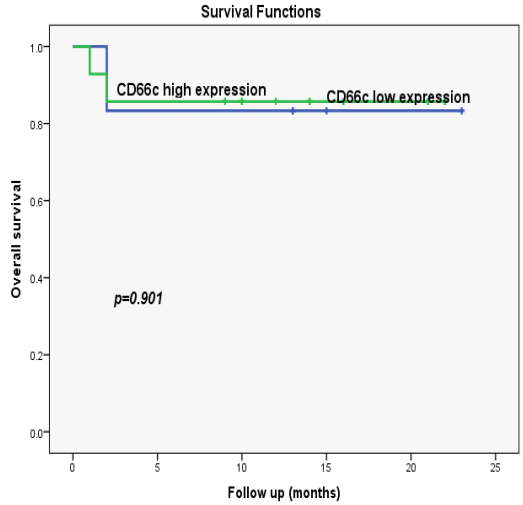

Figure (4): Overall survival of all studied participants according to CD66c level of expression

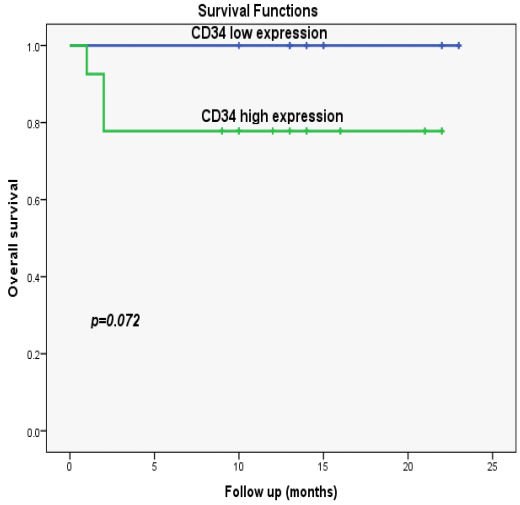

Figure (5): Overall survival of all studied participants according to CD34 level of expression

Discussion:

Acute lymphoblastic leukemia is progressive cancer in children and adults. Malignant transformation and proliferation of the lymphoid progenitor cells in the bone marrow, blood and extra-medullary sites produce the disease. Precursor B-cell type represents most of ALL cases, but the T-cell neoplasm is a rare and extremely aggressive phenotype and is slightly more common in adults than in children [14]. MRD is emerging as the most important predictor of prognosis in the treatment of acute lymphoblastic leukemia. MRD studies in hematology laboratories are an extremely important and challenging task. There is a need to find new markers and to increase MRD sensitivity by using the most sensitive and low-cost markers without compromising the results [15].

This prospective study was held in SECI in the duration from January 2019 to December 2020 on sixty patients (n=60) to evaluate CD66c for MRD by flow cytometry in B-ALL patients and to assess the effect of this marker on the treatment outcomes and overall survival of patients in the study.

Gender:

The patients in this study were 36 (60%) males and 24 (40%) females with a male to female ratio of 3:2. This was in accordance with previous studies by [16] who confirmed a male predominance in ALL patients.

Correlations between markers and blood picture parameters:

Considering CD66c, we found no significant correlations between the mean level of expression of CD66c and WBCs count, PB, or BM blast cell percentage. In concordance with our results, the results of [13] studied 365 children with B-ALL and concluded that there was no correlation between WBCs count at diagnosis and CD66c level of expression.

Level of expression of markers at diagnosis:

CD66c: Similar to our study, [17] studied (27) B-ALL cases for expression of CD66c where high expression was observed in 14/27 cases (58.1%) with a mean value of 31.1%±32.8% and median value of 23% ranging from 0.0% to 93%. They recommended CD66c as a discrimination marker between malignant B-ALL and normal B-cell precursors. Also, [18] confirmed that the recent addition of CD66c in the EuroFlow techniques provided valuable information by the separation obtained of malignant populations. On the contrary of our results, [19] studied the frequency of expression of CD66c in adult B-ALL (n = 43; male n = 20; female n =23 with a median age of 38 y). They found that overexpression of CD66c was observed in 35/43 cases (81.4%). They confirmed the stable expression of CD66c and recommended its use in recognition of abnormal leukemia cells at primary diagnosis and in monitoring of MRD during treatment. Also, [20] analysed 73 cases (median age 22y), (33 males and 40 females) for the expression of CD66c where over-expression was observed in 52.17% of cases. These differences may be explained by different age groups between our study and these studies as well as ethnic variations.

CD34:

In our study, CD34 overexpression was observed in number of cases (67.5%) with mean value 38.48% ± 29.65% and median value 47.8% ranging from 0.4% to 81.5%.

As in our analysis, [21] tested 335 pediatric ALL cases for the clinical Significance of CD34 expression and found that CD34 antigen was expressed in 235 (70%) of cases.

Level of expression of markers at post-induction phase:

At post induction phase, markers were evaluated for the stability of their surface expression from diagnosis to relapse. This means that no complete loss or gain of markers could be found.

CD66c:

At post induction phase, CD66c showed highest stability as cases with overexpression were 12 out of 28 positive cases at diagnosis (42.9%). Also, the mean value for CD66c in patients in CR was 35.01% ±32.31% in comparison to 47.89% ±32.62% in relapsed patients ($p<0.05$).

In agreement with our results, [20] studied twelve cases post induction bone marrow aspirate samples and analysed them for the stability of CD66c and they were found to be stably expressed. Also, [13] followed up 39 childhood cases from diagnosis to relapse and concluded that CD66c expression stays qualitatively stable from diagnosis to relapse in all relapsed cases studied. This finding together with high frequency of CD66c positive cases can support inclusion of CD66c into panels for MRD detection in patients positive for this marker at diagnosis.

CD34: At post induction phase, CD34 showed that cases with overexpression were 4 out of 27 positive cases at diagnosis (14.8%). Also, the mean value for CD34 in patients in CR was 30.02% ±25.49% in comparison to 60.77% ±29.23% in relapsed patients ($P= 0.00$).

In agreement with our study, [22] concluded that CD34 pattern in B-ALL cannot be used as specific surface marker. However, CD34 can serve as specific biomarker for prognosis. Lack of CD34 expression is associated with favorable prognosis.

Correlations between markers and overall survival:

CD66c: we found no significant difference between low and high level of expression of CD66c regarding OS in agreement with [17] where their study included 33 newly diagnosed B-ALL cases of both sexes. The median follow-up period for B-ALL cases in their study was 31.8 weeks and they found no significant correlation between OS and CD66c.

CD34: Also, no significant difference was found between low and high level of expression of CD34 regarding OS. In disagreement with our results, [22] observed that CD34 was more expressed in poor-risk B-ALL adult patients and lack of CD34 is associated with better overall survival rates. The difference may be due to inclusion of children and adults in our studied participants with the majority of our patients were children (88.3%).

Conclusion:

CD66c is highly expressed in our B-ALL patients and is stably expressed after induction of treatment, so its addition in MRD panels can contribute to increasing the sensitivity of the assay. As regard its prognostic value, we couldn't precise its use for evaluation of overall survival in B-ALL patients.

Recommendations:

- The addition of CD66c in routine MRD panels can contribute in increasing the sensitivity of the assay in B-ALL patients.
- A large cohort study of B-ALL cases is recommended.
- The combination of CD66c, CD73, CD86 and CD304 in MRD panels can result in a powerful strategy to identify residual clones in B-ALL patients.
- A combination study between flowcytometry (using CD66c) and PCR MRD techniques could be of great significance in MRD.

Competing Interests:

There are no competing interests.

Authors' Contributions:

M.R. have carried out the preparation of samples, acquisition of data, analysis and interpretation of data and drafted the manuscript. N.S. & A.M. have contributed to designing the work. R.B. has contributed by supervising and revising the work. All authors revised and approved the final manuscript.

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