International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

CD 80 and CD 86 expressions in childhood acute lymphoblastic leukemia

Reem Yehia Abdelazeem Elgarhy[1], Khaled Mohamed Salah[1], Waleed Mahmoud Abdelhameed[1], Mostafa Ahmed Elsayed[1], Nada Hussein Ali Sholkamy[2], Nagwa Ismail Okaily Diab[1]

[1]*Clinical Pathology Department-Faculty of Medicine -Minia University, Egypt.*
[2]*Clinical Oncology and Nuclear Medicine - Faculty of Medicine -Minia University, Egypt.*

Abstract

Acute lymphoblastic leukemia (ALL) is a malignant disorder of lymphoid progenitor cells. Some of the most used therapeutic development for acute leukemia depend on the involvement of costimulatory pathways and molecules including CD80 and CD86. The study carried out on 20 children newly diagnosed as acute lymphoblastic leukemia (group Ia) and were followed up after induction therapy on day 14 (group Ib) to assess response to therapy. Each child was subjected to complete history taking, clinical examination, laboratory investigations in the form of routine investigations; CBC, LDH. Bone marrow aspiration, Immunophenotyping on bone marrow samples determined by Flowcytometer for routine panel of acute leukemia in addition to CD80 and CD 86 expressions on blast cells. CD80 and CD86 expressions were statistically significant decreased in group Ib after induction therapy than group Ia (P- value = 0.04, P- value < 0.001) respectively. Before induction therapy there were no statistically significant correlation between CD80 % and other studied parameters , however Significant positive correlation between CD86% and total leucocytic count , bone marrow blast cells % , peripheral blood blast cells % and LDH level (r = 0.67, p-value = 0.002& r = 0.62, P- value = 0.004& r = 0.5, p-value = 0.027& r = 0.73, P- value < 0.001) respectively, statistically significant negative correlation between CD80% and hemoglobin(HB) level (r = - 0.63, p-value = 0.003). After induction therapy (there was statistically significant positive correlation between CD 80% and BM blast cells % (r = 0.68, p value = 0.003). Statistically significant negative correlations between CD 86% and Hb level (r = - 0.62, p-value = 0.005). Statistically significant positive correlation between CD86% and TLC, BM blast cells%, peripheral blast cells% and LDH level (r = 0.49, p-value = 0.034& r = 0.73, p-value = 0.001& r = 0.77, p-value < 0.001& r = 0.63, p-value = 0.004) respectively. CD86 and CD80 expressions act as valuable markers for managing ALL. Their clinical applications can guide treatment strategies for this disease.

Keywords: ALL, costimulatory molecules, CD 80, CD86.

Full length article **Corresponding Author*, e-mail: nagwalab1980@gmail.com

1. Introduction

Acute lymphoblastic leukemia (ALL) is an aggressive form of leukemia characterized by malignant lymphocytes in the bone marrow. ALL comprises a heterogeneous group of diseases with different morphologic, cytogenetic, and molecular subgroups, some of which carry significant therapeutic implications [1]. Acute lymphoblastic leukemia is seen in both children and adults, but its incidence peaks between 2 and 5 years and also increases in the older population. Although most children can be cured [2]. Several factors at diagnosis, both clinical and biological, influence a patient's outlook and response to treatment in ALL. These include age, gender, bone marrow involvement, blood cell counts, chromosome abnormalities, spread beyond the bone marrow, and immune characteristics. Immune responses of T cells against tumors first involve recognition by the T cell receptor (TCR) or tumor antigen-derived peptides in association with the MHC I antigens. For the T cell to acquire the functions of an effector cell a second transmembrane signal event is required. This second signal is provided by costimulatory molecules, which are expressed on APCs as dendritic cells and certain macrophages and bind to the ligands of the costimulatory molecules that are expressed on T cells. Two of these costimulatory molecules are CD80 (B7-1) and CD86 (B7-2) [3]. CD 80, which is alternatively called B7.1, is a coregulatory receptor expressed on the activated T cells, dendritic cells, macrophages, monocytes which are seen on the surface of antigen presenting cells [4]. It can also be found on the myeloid-derived suppressor cells [5]. Previous investigations unveiled the importance of CD28/B7 family of coregulatory receptors, and that CD 80 is

associated with regulation of the immune system [6]. Hence, it is capable of enhancing tumor progression in the tumor microenvironment by impeding the immune response of the tumor [7]. Cluster of Differentiation 86 (also known as CD86 and B7-2) is a protein expressed on dendritic cells, macrophages, B-cells, and other antigen-presenting cells. Some of the most used therapeutic developments for acute leukemia depend on the involvement of costimulatory pathways and molecules including CD86 [8]. The expression of CD 86 is low and upregulated quickly on the APC while that of CD 80 is inducible expressed later than CD 86. The costimulatory molecules on APCs have been investigated extensively meanwhile their functions on the T cells are yet to be elucidated [7]. The expressions of CD80, CD86 on ALL cells from newly diagnosed patients were variable [9].

2. Materials and Methods

2.1. Ethical consent

This study was approved by Academic and Ethical Committee of Minia University. Providing written informed consent from participants (Approval number : 95:9/2021, Date of approval : 27 September 2021) . All procedures in this study have been performed in compliance with the principles in the World Medical Association's Declaration of Helsinki on human research ethics.

2.2. Patients

This study was carried out at the Clinical Pathology Department, Oncology and Pediatric department Faculty of Medicine, Minia University, Egypt, through the period from November 2021 to June 2022. This study was conducted on 20 children; Group Ia: included 20 newly diagnosed children with acute lymphoblastic leukemia (their ages ranged from 5 to 11 years and included 10 males and 10 females). Group Ib: The same 20 patients after induction therapy on day 14. The patients were subjected to the following: complete history taking, clinical examination, abdominal ultrasonography and laboratory investigations All clinical data were extracted from the hospital information system and patients' paper medical records. Patients received induction therapy & were followed up on day 14 to assess response to therapy by clinical evaluation as well as CBC.

Inclusion criteria

Patients newly diagnosed with ALL

Exclusion criteria

- Patient refusal
- Patients diagnosed with hematological malignancy other than ALL

2.3. Blood sampling protocol

About 4.0 ml of venous blood samples were withdrawn from each subject under complete aseptic conditions: - 2 ml of blood was evacuated in Ethylene Diamine Tetra acetic Acid (EDTA) containing tube for CBC - 2 ml in plain tube, Blood was left to be clotted for 30 min in the incubator then centrifuged at 3000 rpm for 15 minutes. The expressed serum was used for determination of LDH.

2.4. Routine investigations

Complete blood count determined by CELLTAC G, NIHON KOHDEN CORPORATION AUTOMATED HEMATOLOGY ANALYSER, (Japan). Examination of Leishman-stained peripheral blood smears for differential leucocytic count. Bone marrow aspiration and, assessment of blast cell number and morphology. Marrow puncture needles (Klima type) and examination of leishman-stained smears did bone marrow aspiration. , LDH determined by SELECTRA PRO XL, ELITech group, clinical chemistry automation systems , Netherlands, using the commercially available kits according to manufacturer's instructions.

2.5. Special investigations

Immunophenotyping was done on bone marrow samples determined by Flowcytometer (BD FACS canto II, USA) for routine panel of acute leukemia, CD80 and CD86 expressions .

2.6. Staining procedure for CD 80 and CD 86 expressions

For each sample, two tubes were labeled 1&2, one was the test tube and the other tube was used for isotypic control then Hundred ul (40 ul) of bone marrow samples were added in both tubes then 20 ul of phycoerythrin (PE) conjugated anti-CD80 and Fluorescein isothiocyanate (FITC) conjugated anti-CD86 were added to the first tube (test tube). Then both tubes were vortexed and then incubated in the dark at room temperature for 15-20 minutes and then 2 ml of lysing buffer solution were added to each tube then tubes were vortexed and incubated for 10 minutes at room temperature in the dark. Then the tubes were centrifuged at 1200 rpm for 5 minutes and the supernatant was discarded and then 2 ml phosphate buffer solution (PBS) was added to each tube and mixed well then the tubes were centrifuged at 1200 rpm for 5 minutes and then supernatant was discarded. Finally, cells were suspended in 300 ul PBS, then tubes were ready for acquiring data by flow cytometric analysis. Gating was done on the blast cell population based on forward (cell-size) and side scatter (granularity) properties, and CD80 and CD86 expression were assessed using flowcytometry (BD FACS canto II, USA). Data processing was carried out with the Diva software. The analysis of the data was carried out by using IBM SPSS 26.0 statistical package software (IBM; Armonk, New York, USA). Expression of data was as mean, standard deviation (SD), minimum and maximum of range for quantitative measures . Independent sample t test for non-parametric data used for comparison between the two independent groups. To compare categorical variables ,the Chi-square test or Fisher's exact test were used. A p-value less than 0.05 was considered significant.

3. Results and discussion

This work included 20 patients diagnosed as ALL and followed up after induction therapy. Group Ia: ALL 20 patients before start of induction therapy and group Ib: the

same 20 patients after induction therapy. Statistically significant decreased Hb level , TLC, BM blast cells % , peripheral blast cells % , LDH level , CD80% and CD 86% in group Ib after induction therapy than in group Ia in ALL patients (p-value = 0.002 , p-value < 0.001, p-value < 0.001, p-value < 0.001, p-value < 0.001, p -value = 0.04 , p-value < 0.001) respectively **(**Table 1&figure 1& figure 2). Before induction therapy (No statistical significant correlation between CD80% and other studied data (p-value > 0.05), however. Significant positive correlation between CD86% and total leucocytic count, peripheral blood blast cells%, bone marrow blast cells% and LDH level (r= 0.67, p-value = 0.002& r= 0.5, p-value = 0.027& r=0.62, P- value=0.004 & r=0.73, P- value < 0.001) respectively. However, there was statistically significant negative correlation between CD80% and hemoglobin (HB) level (r = - 0.63, p-value = 0.003) (table 2&table 3). After induction therapy (there was statistically significant positive correlation between CD80% and BM blast cells% (r = 0.68, p value =0.003). Statistically significant negative correlation between CD 86% and Hb level (r = - 0.62, p-value = 0.005). Statistically significant positive correlations between CD86% and TLC, BM blast cells%, peripheral blast cells% and LDH level (r = 0.49, (p-value = 0.034& r = 0.73, p-value = 0.001& r = 0.77, p-value < 0.001& r = 0.63, p-value = 0.004) respectively (tables 4, 5 &figures 3, 4). Acute lymphoblastic leukemia (ALL) is the most common hematological malignancy in childhood and accounts for about 20% of acute leukemia in adults [10]. Recognition of CD86 ligand by costimulatory CD28 and co-inhibitory CTLA-4 receptors plays an important role in influencing immune responses by proliferation and suppression of effector T cells respectively [11]. CD86 is expressed on the surface of antigen presenting cells (APCs) as monocytes and dendritic cells (DCs), its expression was found to be associated with many hematological malignancies such as acute myeloid leukemia (AML) and it was reported as a marker of poor prognosis in it [12]. A number of clinical and biological factors at the time of presentation are relevant to the prognosis and affect the response to treatment. These prognostic factors include age, number of blasts, white blood cells (WBC) count, platelet count, cytogenetic abnormalities, extra medullary involvement (EMI) and immune phenotype [13]. The study showed significant decrease in hemoglobin level, and this was in agreement with Qian et al., 2021 [14] who showed that the hematologic adverse effects, including thrombocytopenia, leukopenia and anemia, are the most frequent events for treatment. About WBCs count**,** Topp et al., 2015 [15] showed that High WBC count at diagnosis is also an adverse factor, with patients presenting with WBC count≥50,000/mm3 having worse outcome. That was similar to our study as we found statistically significant positive correlation between CD86 and TLC. Regarding the blast cell count, our study showed highly statistical significant decreased peripheral blast cells% after induction and this was similar to Park et al ., 2016 [13] that showed in their studies that those with a good response to steroid, that is a peripheral blast count have better survival compared with those with a poor response to steroid. About thrombocytopenia in patients, this was in agreement with Sharabi et al ., 2018 [16] who showed its pathogenesis and association with ALL. Regarding LDH, our study showed highly statistical significant decreased LDH after treatment when compared with LDH before treatment in ALL patients. These observations were explained as the patients with ALL on presentation had high LDH levels and with the initiation of induction therapy enzymatic activities dropped gradually till normalization this was coincidental with clinical and hematological remission this was in agreement with Zahra et al., 2021 [17] who showed that the serum. LDH level was highly elevated at diagnosis in the majority of ALL patients decreased significantly in response to chemotherapy. Regarding CD 86 and CD 80 expressions, our study revealed statistically decreased CD80 and CD 86 expressions after treatment when compared with CD80 and CD86 before treatment in ALL patients. CD86 was positively expressed in newly diagnosed patients, this finding was in agreement with Mansour ***et*** al. 2014 [3] who reported that CD86 was positively expressed in 65% of the studied ALL patients. According to the relationship between CD86 expression and various studied standard prognostic factors, there were significant positive correlations between CD86 and LDH level, white blood cells in ALL patients. However, there were significant negative correlations between CD86 expression and both hemoglobin level and platelet count. CD80 and CD86 expressions were significantly positively correlated with bone marrow blast cells. This was in agreement with Ahmed and Hassab, 2008 [18] who made their study on 30 newly diagnosed ALL pediatric patients for CD80 and CD86 expression by flow cytometry and correlated it to age, sex, TLC, HB, platelet count, blast % in peripheral blood and bone marrow, immunophenotyping and LDH. In contrary with our study, Brudno et al., 2016 [19] showed another explanation of the statistically significant positive correlation between both of CD80 and CD86 expressions and the percentage of the blast cells that we found. They showed that the expressions of CD86 and CD80 on ALL blasts (leukemia cells) are variable and can be high and that is associated with better prognosis as it facilitates efficient T cell-mediated killing of leukemia cells or low and that indicates impaired T cell activation and potentially worse prognosis. It is important to note that CD86 and CD80 expressions alone are not conclusive for diagnosis or prognosis. They should be considered in conjunction with other clinical and laboratory findings and molecular studies. More researches are needed to fully understand the complex interplay between CD86/CD80, T cells, and the immune response in ALL.

Table 1. Comparison of studied data before and after induction therapy in ALL patients.

ALL group		Group Ia (N = 20)	Group Ib (N = 20)	P-value
Hb (g /dl)	Range	7.1 –12.3	6–10	0.002*
	Mean ±SD	9.1 ± 1.6	8.2± 1.2	
TLC (x10³/ul)	Range	19– 53	2– 7	< 0.001*
	Mean ±SD	35.3 ± 11.8	4.2 ± 1.4	
PLTs (x10³/ul)	Range	22– 69	22– 70	0.094
	Mean ±SD	37.7 ± 12.4	47.4 ± 14.7	
BM Blast cells (%)	Range	22– 87	1– 22	< 0.001*
	Mean ±SD	41.7 ± 21.9	7.6± 6.6	
P. Blast cells (%)	Range	15– 31	1– 6	< 0.001*
	Mean ±SD	22.8 – 5.3	3.3 ±1.7	
LDH (U/L)	Range	440– 990	170– 720	< 0.001*
	Mean ±SD	655.1± 199.3	368.1 ± 179.4	
CD80%	Range	1.1– 7.9	0.8 –5	0.04 *
	Mean ±SD	2.9 ±1.7	2.2.±1.1	
CD 86%	Range	4.3– 55	1.9– 26	< 0.001*
	Mean ±SD	34 ± 17.8	12.2 ± 8.1	

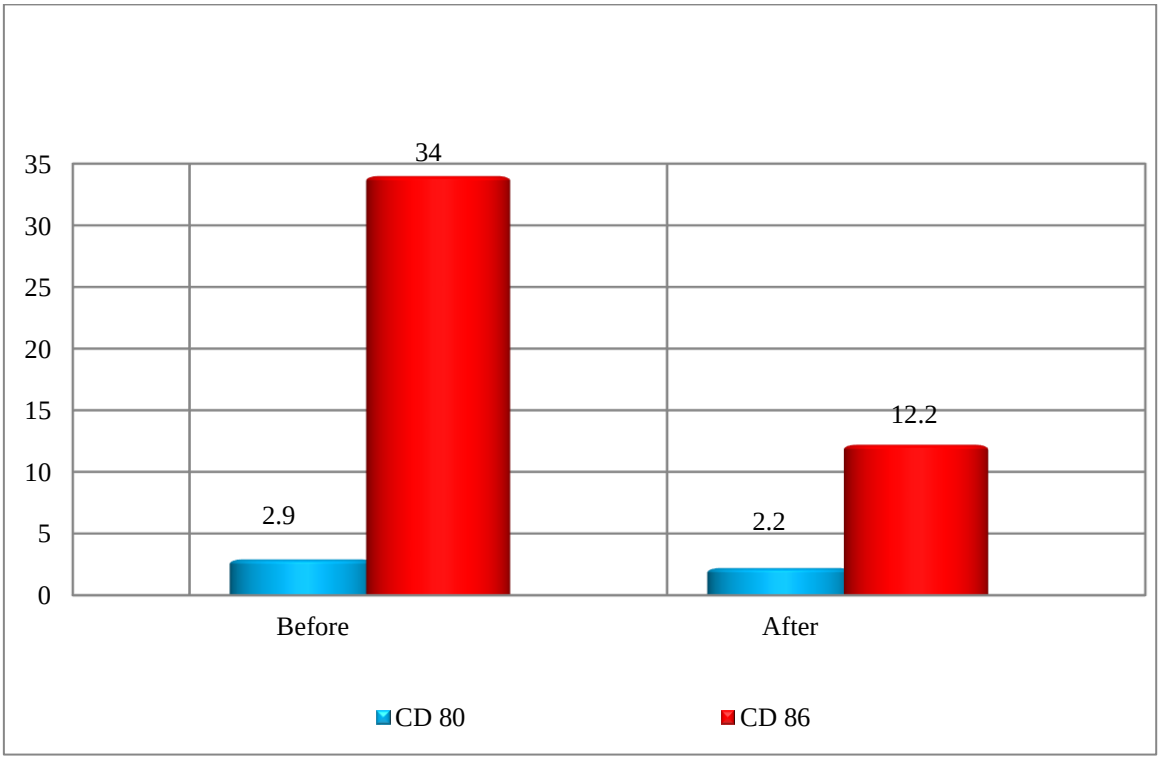

Figure 1. Comparisons of CD80 & CD86 expressions before and after induction therapy in ALL patients

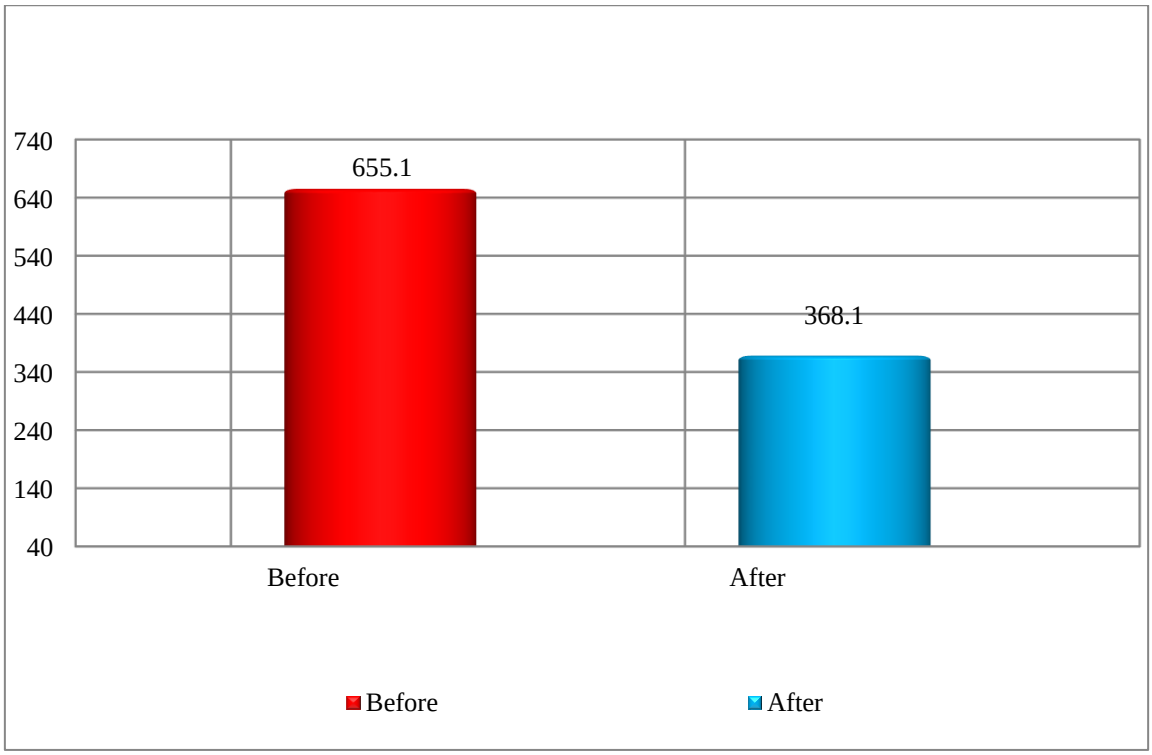

Figure 2. Comparisons of LDH before and after induction therapy in ALL patients.

Table 2. Correlations between CD 80% and other studied data (before induction therapy)

CD 80% (Before induction therapy)	ALL	
	R	p-value
CD 86%	0.23	0.346
Hb (g/dl)	-0.44	0.058
TLC (x10³/ul)	0.35	0.138
PLTs (x10³/ul)	0.30	0.219
BM Blast cells%	0.08	0.752
Absolute Lymphoctic count (/ul)	0.39	0.098
Peripheral blast cells%	0.29	0.221
LDH(U/L)	0.40	0.094

Table 3. Correlations between CD 86% and other studied data (before induction therapy)

CD 86% (Before induction therapy)	ALL	
	R	p-value
CD 80%	0.22	0.346
Hb (g/dl)	- 0.63	0.003*
TLC (x10³/ul)	0.67	0.002*
PLTs (x10³/ul)	0.37	0.117
Bone marrow Blast cells%	0.62	0.004*
Absolute Lymphocytic count (/ul)	0.43	0.061
Peripheral blast cells%	0.5	0.027*
LDH (U/L)	0.73	< 0.001*

Table 4. Correlations between CD 80% and other studied data (after induction therapy) in ALL studied groups

CD80% (After induction therapy)	ALL	
	R	p-value
CD 86%	0.45	0.056
Hb (g/dl)	-0.06	0.818
TLC(x10³/ul)	-0.04	0.876
PLTs (x10³/ul)	-0.20	0.407
Bone marrow Blast cells %	0.68	0.003*
Peripheral blast cells%	0.34	0.149
LDH (U/L)	0.26	0.286

Table 5. Correlations between CD 86 % and other studied data (after induction therapy)

CD86% (After induction therapy)	ALL	
	R	p-value
CD 80%	0.45	0.056
Hb (g/dl)	-0.62	0.005*
TLC (x10³/ul)	0.49	0.034*
PLTs (x10³/ul)	-0.15	0.542
BM Blast cells %	0.73	0.001*
Peripheral blast cells %	0.77	< 0.001*
LDH (U/L)	0.63	0.004*

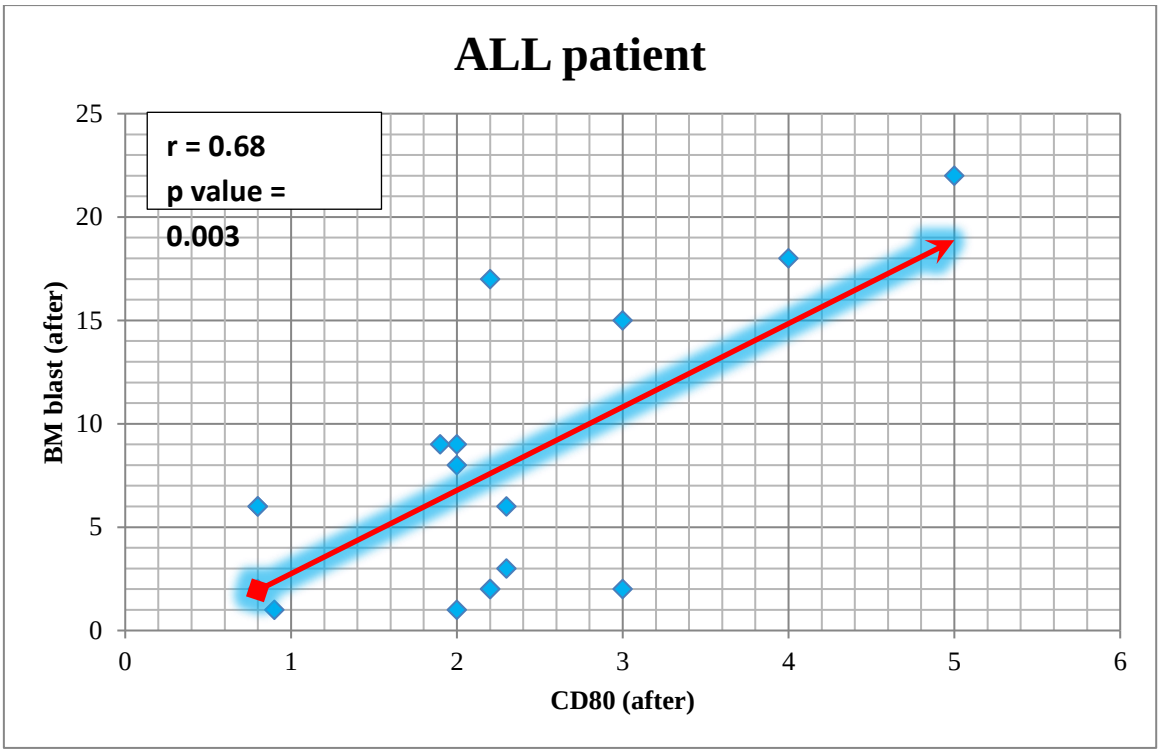

Figure 3. Positive correlation between CD80% and BM blast cells% (after induction therapy) in ALL patients.

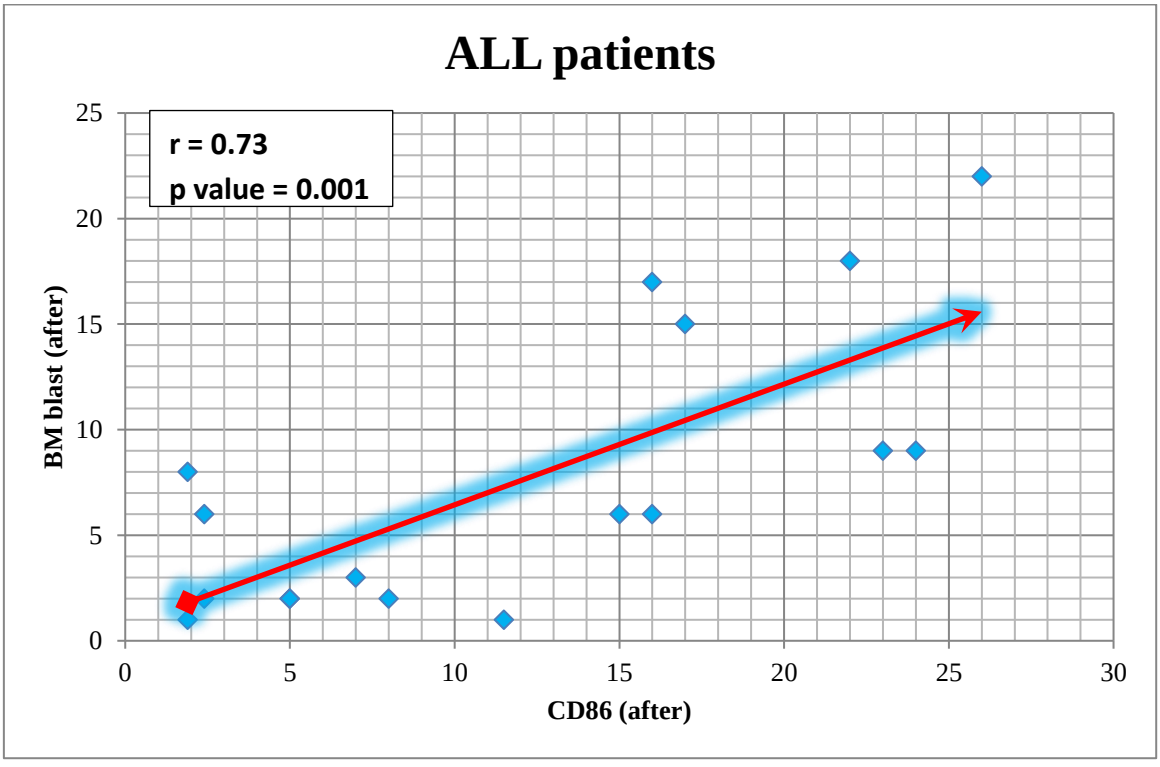

Figure 4. Positive correlation between CD 86% and BM blast cells% (after induction therapy) in ALL patients.

4. Conclusions

In conclusion, CD86 and CD80 expressions hold significant promise as valuable markers for understanding and managing ALL. Their clinical applications are actively being explored, with the potential to improve diagnosis, predict response to chemotherapy, and guide treatment strategies for this challenging disease.

References

[1] K. Imai. (2017). Acute lymphoblastic leukemia: pathophysiology and current therapy. [Rinsho Ketsueki] The Japanese Journal of Clinical Hematology. 58 (5) 460-470.

[2] L. Muffly, P. Kebriaei. (2020). Philadelphia chromosome positive acute lymphoblastic leukemia in adults: therapeutic options and dilemmas in 2020. In Seminars in hematology. 57 (3) 137-141.

[3] A. Mansour, T. Elkhodary, A. Darwish, M. Mabed. (2014). Increased expression of costimulatory molecules CD86 and sCTLA-4 in patients with acute lymphoblastic leukemia. Leukemia & lymphoma. 55 (9) 2120-2124.

[4] R.J. Greenwald, G.J. Freeman, A.H. Sharpe. (2005). The B7 family revisited. Annu. Rev. Immunol. 23 515-548.

[5] I. Poschke, D. Mougiakakos, J. Hansson, G.V. Masucci, R. Kiessling. (2010). Immature immunosuppressive CD14+ HLA-DR−/low cells in melanoma patients are Stat3hi and overexpress CD80, CD83, and DC-sign. Cancer research. 70 (11) 4335-4345.

[6] T.J. Curiel, S. Wei, H. Dong, X. Alvarez, P. Cheng, P. Mottram, W. Zou. (2003). Blockade of B7-H1 improves myeloid dendritic cell–mediated antitumor immunity. Nature medicine. 9 (5) 562-567.

[7] M.J. Butte, M.E. Keir, T.B. Phamduy, A.H. Sharpe, G.J. Freeman. (2007). Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. Immunity. 27 (1) 111-122.

[8] A.M. Abdel-Razik, M.A. Wahba, H.M. El-Maraghy. (2018). CD86: A Novel Prognostic Marker in Acute Lymphoblastic Leukemia Patients. The Egyptian Journal of Hospital Medicine. 71 (6) 3373-3377.

[9] M. Yilmaz, H. Kantarjian, F. Ravandi-Kashani, N.J. Short, E. Jabbour. (2018). Philadelphia chromosome-positive acute lymphoblastic leukemia in adults: current treatments and future perspectives. Clin Adv Hematol Oncol. 16 (3) 216-223.

[10] E. Liew, E.G. Atenafu, A.D. Schimmer, K.W. Yee, A.C. Schuh, M.D. Minden, J.M. Brandwein. (2012). Outcomes of adult patients with relapsed acute lymphoblastic leukemia following frontline treatment with a pediatric regimen. Leukemia Research. 36 (12) 1517-1520.

[11] M.A. Postow, M.K. Callahan, J.D. Wolchok. (2015). Immune checkpoint blockade in cancer therapy. Journal of clinical oncology. 33 (17) 1974.

[12] A.J. Barrett, K. Le Blanc. (2010). Immunotherapy prospects for acute myeloid leukaemia. Clinical & Experimental Immunology. 161 (2) 223-232.

[13] H.J. Park, E.K. Moon, J.Y. Yoon, C.M. Oh, K.W. Jung, B.K. Park, Y.J. Won. (2016). Incidence and survival of childhood cancer in Korea. Cancer research and treatment: official journal of Korean Cancer Association. 48 (3) 869-882.

[14] Q. Cui, C. Qian, N. Xu, L. Kang, H. Dai, W. Cui, X. Tang. (2021). CD38-directed CAR-T cell therapy: a novel immunotherapy strategy for relapsed acute myeloid leukemia after allogeneic hematopoietic stem cell transplantation. Journal of Hematology & Oncology. 14 (1) 1-5.

[15] M.S. Topp, N. Gökbuget, A.S. Stein, G. Zugmaier, S. O'Brien, R.C. Bargou, H.M. Kantarjian. (2015). Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multicentre, single-arm, phase 2 study. The Lancet Oncology. 16 (1) 57-66.

[16] A. Sharabi, M.G. Tsokos, Y. Ding, T.R. Malek, D. Klatzmann, G.C. Tsokos. (2018). Regulatory T cells in the treatment of disease. Nature reviews Drug discovery. 17 (11) 823-844.

[17] S.S.A. Zahra, E.H. Al-Shammary, I.M. Hameed. (2021). Serum lactate dehydrogenase level in childhood acute lymphoblastic leukemia. Iraqi Journal of Hematology. 10 (1) 55-58.

[18] F. Hassab. (2008). Study of soluble CD44 and its expression by mononuclear cells in children with acute lymphoblastic leukemia: its relation to prognostic factors. Egyptian journal of immunology. 15 (2) 101-111.

[19] J.N. Brudno, R.P. Somerville, V. Shi, J.J. Rose, D.C. Halverson, D.H. Fowler, J.N. Kochenderfer. (2016). Allogeneic T cells that express an anti-CD19 chimeric antigen receptor induce remissions of B-cell malignancies that progress after allogeneic hematopoietic stem-cell transplantation without causing graft-versus-host disease. Journal of clinical oncology. 34 (10) 1112.