Journal of Recent Advances in Medicine

JRAM
Journal of Recent Advances in Medicine
مجلة التطورات الحديثة في الطب
Print ISSN 2636-252X - Online ISSN 2636-2538

Original Article

The Role of immunoregulator CD200 and CD47 in childhood acute lymphoblastic leukemia

Clinical Pathology

Esraa A. Ayad [1], Reham H. Mahmoud [1], Randa A. Osman[2], Mona H. Alrayes[1]

[1] Clinical Pathology Department, Faculty of Medicine for Girl, Cairo, Al Azhar University, Egypt.

[2] Clinical Pathology Department, National Cancer Institute, Cairo University, Cairo, Egypt.

ABSTRACT

Background: About 30% of all pediatric cancers are acute lymphoblastic leukemia (ALL). Leukemia cells capacity to avoid immune system destruction contributes to the disease's onset and spread. One of the adaptive immune response's regulators is CD47. The immunoglobulin superfamily inhibitory receptor CD200R, which is mostly expressed on myeloid/monocyte lineage cells, and a subset of T cells, interacts with CD200. CD200R inhibits immunological responses mediated by T cells and monocytes. The existence of post-therapeutic leukemia cells in the bone marrow is known as minimal residual disease (MRD).

Aim of the study: To identify the role of CD200 and CD47 in pediatric ALL and highlight their possible role in MRD.

Methods: A hospital based cross sectional comparative study was carried out on 52 childhoods ALL cases from both B cell and T cell types. The expression pattern of CD200 and CD47 on blasts was assessed by flow cytometry. The MRD was detected on day 42. The cut of value of MRD is 0.01%.

Results: CD200 blast expression was significantly upregulated in B-ALL group compared to T-ALL group (P =0.017). There was no significant difference in CD47 blast expression between B-ALL and T-ALL (P=0.225). There was a statistically significant increase in hepatomegaly, splenomegaly, para-aortic lymphadenopathy and interior mediastinal mass in T-ALL group compared to B-ALL group (P=0.026, P=0.011, P=0.042 and P < 0.001) respectively.

Conclusion: T-All cases were presented with more metastatic course. B-ALL patients presented initially with significant increase in CD200 expression on blasts. There was no significant difference in CD47 blast expression between B-ALL and T-ALL. No significant difference was shown in MRD levels on day 42 between B-ALL and T-ALL patients

JRAM 2025; 6 (1): 77-83

Key words: CD47; CD200; acute lymphoblastic leukemia.

Submission Date: 5 March 2025 **Acceptance Date:** 8 April 2025

Corresponding author: Esraa A Ayad, Clinical pathology department, faculty of medicine for girls, Al-Azhar university, Cairo, Egypt. **Tel:** +20124559978. E-mail: Esraaayad920@yahoo.com

Cite: Ayad EA, Hammad RM, Osman RA, Alrayes MH. The Role of Immunoregulator CD200 and CD47 in Childhood Acute Lymphoblastic Leukemia. JRAM 2025; 6 (1): 77-83. DOI: 10.21608/jram.2025.364954.1280

INTRODUCTION

The development of a significant number of immature lymphocytes is a hallmark of acute lymphoblastic leukemia (ALL), a malignancy of the lymphoid lines of blood cells. ALL is the second most frequent acute leukemia in adults, despite the fact that it affects children 80% of the time [1].

According to immunophenotype, pediatric ALL can be classified as either B- (B-ALL) or T-lymphoid (T-ALL), which make up 85% and 15% of all cases, respectively. Long-standing characteristics used in risk-stratification algorithms for patients with B-ALL were patient age and white blood cells (WBCs) diagnosis [2].

A type-1 cell membrane glycoprotein belonging to the immunoglobulin supergene family, CD200 is found on numerous cancer cells, epithelial cells, and cells with myeloid or lymphoid origin. Over the last 10 years, it has been shown that the interaction between C200 and its receptor(s), CD200R(s), attenuates a range of immunological responses [3]. Almost every cell in the body expresses CD47, also known as integrin-associated protein, at modest levels. By conveying a strong "don't eat me" signal to stop phagocytosis, it plays a crucial part in both autoimmunity and other immune responses. An increasing amount of data indicates that a number of hematological cancers exhibit overexpression of CD47 [4].

Minimal residual disease (MRD) is the term used to describe a population of leukemia cells that survive chemotherapy or radiation therapy and result in the disease recurring. Finding MRD is crucial for forecasting outcomes and figuring out how intensely to apply following treatment strategies [5]. In the current study we aimed to identify the role of CD200 and CD47 in pediatric ALL.

PATIENT AND METHODS

Study design and population: A hospital based cross sectional comparative study was carried out on 52 childhood ALL cases. B –ALL (n=37) and T -ALL (n=15). The Patients were selected from the inpatients of the Pediatric Oncology department, National Cancer Institute (NCI), Cairo University during the period "between" December 2022 till January 2024. Patients were followed up till May 2024.

Inclusion criteria: newly diagnosed cases of B and T –ALL patients, prior to therapy and aged below 18 years.

Exclusion criteria: children with ALL in relapse and who received treatment.

This study was conducted in compliance with Helseinki's declaration, and the approval of the ethics committee of Al-Azhar University of medicine for girls was obtained (Approval No. 2018122001).

All patients were subjected to full history taking and complete physical examination, with assessment of signs relevant to leukemia as hepatomegaly, splenomegaly, lymphadenopathy, gums or skin infiltration, complete blood pictures were performed by Sysmex XN-1000-2A (Sysmex, Kope, Japan. Examination of the peripheral blood (PB) films stained with Leishman's stain for differential leucocytic count and assessment of blast (%) in PB. The minimal residual disease (MRD) was detected at day 42. The cut of value of MRD is 0.01%.

Flow cytometry assay

Two ml of bone marrow aspirate was collected from each patient under complete aseptic conditions into EDTA vacutainer. All samples were stored at room temperature. Analysis was done on samples within 24 hours of collection. The monoclonal antibodies (Mo Abs) used were purchased from Biosciences systems product. 50 µl of adjusted BM sample was placed in the tubes. 5 µl of each of the labeled Mo Abs (CD45, CD47 and CD200) were added to the first tube incubated in the dark for 15 minutes. After RBCs lysis and washing cells were resuspended with 200-400 µL of PBS for acquisition by (Navios EX (Beckman coulter, USA).

Gating strategy

After performing initial gating on the blasts area in the dot plot graph for CD45/SS, the subset of cells expressing CD200 and CD47 inside the blast population was identified, and their proportion was assessed using a quadrant plot histogram. The upper right quadrant was identified as the location of co-expression, with CD47 on the X axis and CD200 on the Y axis as shown in figure 1.

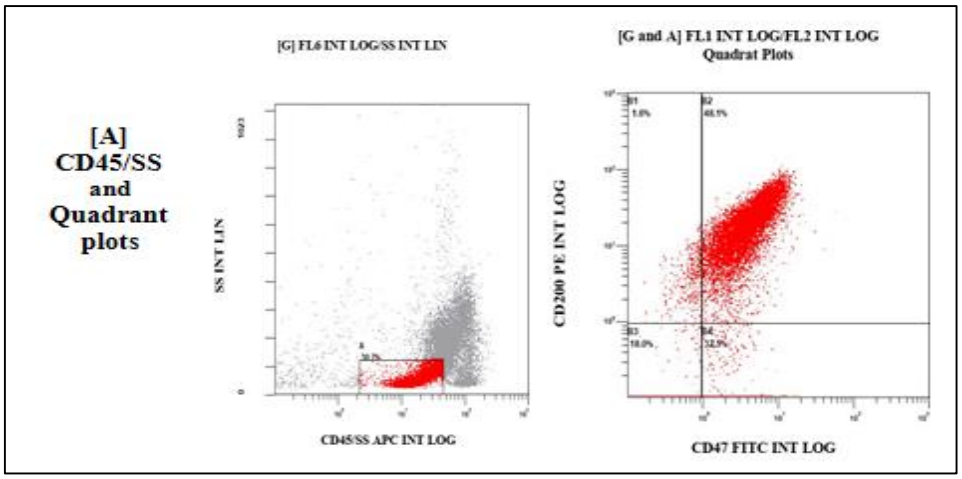

Figure (1): Gating strategy for blast expression of CD200 and CD47

Statistical analysis

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Quantitative variables were summarized as median and interquartile range while categorical data was expressed as frequency (count) and (percentage). For comparing categorical data, Chi square ($\chi 2$) test was performed. The fisher Exact test was used instead when the expected frequency is less than 5**.** Mann Whitney was used for non-parametric quantitative data. Correlations between quantitative variables were done using Spearman correlation coefficient. A p-value less than 0.05 was considered statistically significant.

RESULTS

There was no significant difference between B-ALL and T-ALL groups regarding demographic data. There was a statistically significant increase in hepatomegaly, splenomegaly, para-aortic lymphadenopathy and anterior mediastinal mass in T-ALL group (p=0.026, P=0. 01, p=0.042 and p<0.001, respectively) when compared with B-ALL group. Also, there was no

statistically significant difference in MRD on day 42 in B-ALL group in comparison to T-ALL group (p=0.7) as displayed in table 1.

There was significant elevation of total leucocytic count (TLC) in T-ALL group in comparison to B-ALL group while there was a reduction in platelets count in B- ALL group in comparison to T-ALL group (p=0.001, p=0.035 respectively). The percentage of blasts expressing CD200 was significantly increased in B ALL group compared to T ALL group (p=0.043), There was no significant difference in CD47 blast expression between B-ALL and T-ALL(p=0.225) as displayed in table 2.

Table (1): **Comparison between B ALL group and T ALL group regarding demographic and clinical data**

Variables		B ALL n= 37	T ALL n =15	p value
Age/years	**Median (IQR)**	8.00 (4.00-13.00)	10.00 (5.00-14.00)	0.321
		no. (%)	**no. (%)**	
Sex	**Male**	24 (64.9%)	12 (80.0%)	0.340
	Female	13 (35.1%)	3 (20.0%)	
Hepatomegaly (US)	**Yes**	18 (75.0%)	11 (84.6%)	0.026*
	No	6 (25.0%)	2 (15.4%)	
Splenomegaly (US)	**Yes**	11 (45.8%)	12 (92.3%)	0.011*
	No	13 (54.2%)	1 (7.7%)	
Para aortic LN (US	**Yes**	3 (12.5%)	6 (46.2%)	0.042*
	No	21 (87.5%)	7 (53.8%)	
Anterior mediastinal mass	**Yes**	0 (0.0%)	9 (69.2%)	< 0.001*
	No	22 (100%)	4 (30.8%)	
MRD (day 42)	**Positive**	9 (26.5%)	2 (16.7%)	0.7
	Negative	25 (73.5%)	10 (83.3%)	

IQR: interquartile range; US: ultrasound; LN: lymphadenopathy; *p<0.05 is significant, MRD; minimal residual disease. Tests of significant: Chi square ($\chi 2$) test for qualitative data and Mann Whitney was used for non-parametric quantitative data. NB: Not all cases done ultra sound to detect previous clinical data, *: Significant p-value (<0.05) for Mann Whitney test Chai-square test.

Table2: **Comparison between B-ALL group and T-ALL group regarding the studied parameters**

Items	B ALL n=37	T ALL n=15	p-value
	Median (IQR)	**Median (IQR)**	
TLC× 10^9/L	22 (4-60)	84.70 (46.5-213)	0.001*
PLT× 10^9/L	50 (21-97)	76 (38-210)	0.035*
% of Initial Blast in PB	**75** (44-86)	76 (46-84)	0.86
% of initial Blast in BM	91 (89-95)	89 (84-92)	0.054
% Of blast expressed CD 47	97 (93.5-99.3)	99.4 (92.1-99.6)	0.225
% Of blast expressed CD 200	88.6 (64.7-97.5)	67.4 (25.7-91.6)	0.043*
% of BM Blasts (day 42)	1 (1-2)	1 (1-1)	0.226
Total follow up time\month	8 (6-12)	8 (5-12)	0.992

TLC: Total leucocytic count, HBG: hemoglobin, PLT: Platelet, PB: Peripheral blood, BM: Bone marrow, MFI: Mean fluorescent intensity, *: Significant p-value (<0.05) for Mann Whitney test.

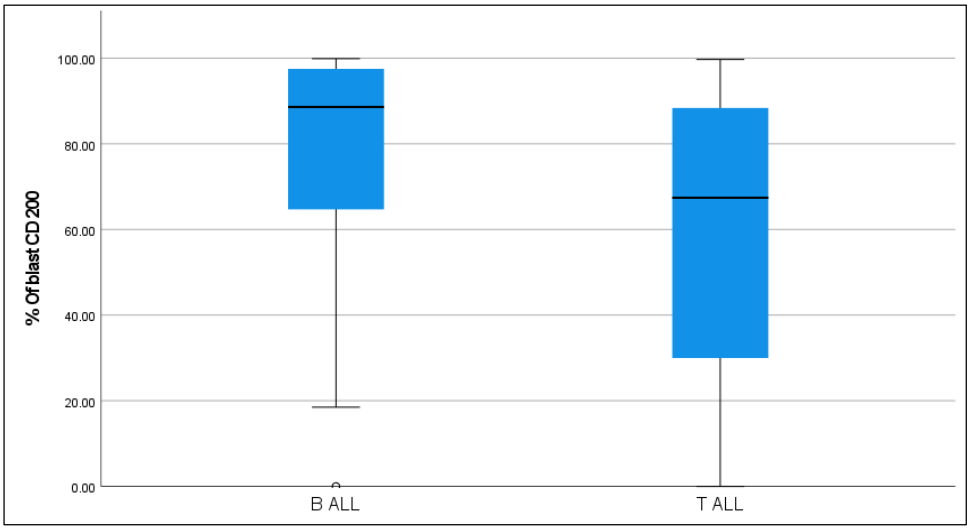

Figure (2): Mean % of blasts expressed CD200 in B-ALL and T-ALL groups

DISCUSSION

In order to prevent immunological harm to stem cells and other vital tissues, CD200 plays a crucial part in immune tolerance. Aref et al. [6] stated that CD200 functions primarily as an immune suppressor, potentially enabling leukemic cells to evade immune detection. The CD47 inhibits macrophage activity by interacting with macrophage receptors, allowing leukemic cells to avoid destruction. CD47 has been reported to influence the tumor microenvironment by promoting M2 polarization and Tregs, contributing to the creation of an immunosuppressive microenvironment. Xu et al. [7] stated that the role of immunoregulatory markers such as CD200 and CD47 in ALL provides insights into potential therapeutic targets and disease mechanisms.

As regard age and sex there was no significant difference between B-ALL and T-ALL subgroups at the present work. On the contrary, Van Vlierberghe et al. [8] reported that T-cell ALL appeared to have a 2–3 times higher incidence in male patients than female patients. Fernanda et al. [9] highlighted that the biological differences between B-ALL and T-ALL were driven primarily by genetic and molecular alterations intrinsic to the disease.

The clinical analysis in this study revealed that patients diagnosed with T-ALL exhibited a significantly higher incidence of hepatomegaly, splenomegaly, and para-aortic lymphadenopathy compared to those with B-ALL subtype. These findings could suggest that T-ALL presents with a more extensive extramedullary involvement, contributing to its aggressive clinical course. In agreement, Pui et al. [10] confirmed that T-ALL patients commonly present with higher rates of organ involvement and lymphadenopathy supporting that T-ALL had a more aggressive clinical phenotype compared to B-ALL. While Kakaji et al. [11] reported no significant difference between T-ALL and B-ALL subtypes as regard organomegaly and lymphadenopathy among pediatric Syrian ALL patients.

Also, in the current study there was a high significant increase in presence of anterior mediastinal mass in T-ALL subtype in comparison to B-ALL subtype. Terwilliger and Abdul-Hay[12] reported that T-ALL patients showed diffuse infiltration of the bone marrow by immature T cell lymphoblast and mediastinal enlargement. Heterogeneity in genetic backgrounds, environmental exposures, and the timing of diagnosis may contribute to variations in clinical presentation. Additionally, differences in the sensitivity and specificity of imaging techniques used to detect organ involvement might account for some conflicting results.

In the current study a significant difference was observed in the hematological parameters between patients with B-ALL and T-ALL subtype. Specifically, patients in the T-ALL subgroup exhibited significantly higher TLC compared to those in the B-ALL subgroup. These findings suggest that T-ALL may present with a more aggressive hematological profile at diagnosis. In agreement, Margolin et al.[13] and Vaitkeviciene et al. [14] reported that hyperleukocytosis was usually associated with T-cell immunophenotyping. Also, Chiaretti et al. [15] reported that T-ALL was often associated with hyperleukocytosis which was a well-documented marker of poor prognosis in acute leukemia. Girardi et al. [16] found that T-ALL patients frequently presented with elevated TLC, and this had been correlated with a more aggressive disease course and a higher risk of CNS involvement.

The platelet count was significantly reduced in B-ALL subgroup in comparison with T-ALL subgroup at the present work. On contrary, a study carried by Kakaji et al. [11] on children with ALL at the Children's University Hospital of Damascus University-Syria, the age of the studied children was between 0-14 years, they reported no significant difference between T-ALL and B-ALL in children as regard platelet count.

The percentage of lymphoblasts expressing CD200 was significantly higher in B-ALL subtype in

comparison to T-ALL subtype at the present work. Similarly, Chao et al. [17] reported elevated levels of CD200 in B-ALL. While Tonks et al. [18] did not find significant differences in CD200 expression between B-ALL and T-ALL.

The percent of lymphoblasts expressing CD47 revealed no significant difference between B-ALL and T-ALL subtypes. This was supported by Arai et al. [19] who found that high CD47 expression in colorectal cancer tumor was associated with the activation of several oncogenic pathways and an immune-engaged tumor microenvironment. CD47 was a representative innate immune checkpoint involved in the evasion of tumor cell phagocytosis by macrophages. This finding is consistent with other studies linking CD47 over expression to poor prognosis and resistance to standard therapy Majeti et al. [20]

Patients at the present work were followed up for detection of minimal residual disease (MRD) on day 42 of induction therapy. B-ALL patients showed no significant difference in MRD levels on day 42 compared to T-ALL patients. Khalil et al. [21] demonstrated that the correlation between initial CD200 expression and MRD day 42 showed no statistically significant correlation among B-ALL patients.

CONCLUSION

T-All cases were presented with more metastatic course. B-ALL patients presented initially with significant increase in CD200 expression on blasts. There was no significant difference in CD47 blast expression between B-ALL and T-ALL. Still, no significant difference was detected in MRD levels on day 42 compared to T-ALL patients

Funding: No fund

Conflicts of Interest: The authors declare no conflicts of interest regarding the publication of this paper.

REFERENCES

1. **Jędraszek K, Malczewska M, Parysek-Wójcik K, and Lejman M.** Resistance mechanisms in pediatric B-cell acute lymphoblastic leukemia. Int J Mol Sci. 2022; 12;23(6):3067
2. **Inaba H and Mullighan CG.** Pediatric acute lymphoblastic leukemia. Haematologic. 2020; 105(11):2524.
3. **Kotwica-Mojzych K, Jodłowska-Jędrych B, Mojzych M (2021)**:"CD200:CD200R Interactions and Their Importance in Immunoregulation". International Journal of Molecular Sciences; **22** (4): 1602.
4. **Russ A, Hua AB, Montfort WR, Rahman B, Riaz IB, Khalid M, et al.** Blood Rev. 2018**;**32(6):480-489.
5. **Kruse A, Abdel-Azim N, Kim HN, Ruan Y, Phan V, Ogana H, et al.** Minimal residual disease detection in acute lymphoblastic leukemia. Int J Mol Sci. 2020; 5;21(3):1054.
6. **Aref S, Azmy E, El-Bakry K, Ibrahim L, and Abdel Aziz S.** Prognostic impact of CD200 and CD56 expression in pediatric B-cell acute lymphoblastic leukemia patients. Pediatr Hematol Oncol.2017;34(5),275.
7. **Xu X, Li H, and Xu C (2020):** Structural understanding of T cell receptor triggering. Cellular and Molecular Immunology. 2020; 17(3),193.
8. **Van Vlierberghe P, Palomero T, Khiabanian H, Van der Meulen J, Castillo M, Van Roy N, et al.** PHF6 mutations in T-cell acute lymphoblastic leukemia. Nat Genet. 2010;42(4),338.
9. **Pereira FA, Mirra AP, de Oliveira MD, De Assunção JV. Environmental risk factors and acute lymphoblastic leukaemia in childhood. Ciencias de la salud. 2017;15(1):129-44.**
10. **Pui CH, Pei D, Sandlund JT, Ribeiro RC, Rubnitz JE, Raimondi SC, et al.** Long-term results of St Jude Total Therapy Studies 11, 12, 13A, 13B, and 14 for childhood acute lymphoblastic leukemia. Leukemia. 2010; 24(2),371.
11. **Kakaje, A., Alhalabi, M.M., Ghareeb, A., Karam, B., Mansour, B., Zahra, B. and Hamdan, O., 2020. Rates and trends of childhood acute lymphoblastic leukaemia: an epidemiology study. Scientific reports, 10(1), p.6756.**
12. **Terwilliger T and Abdul-Hay M (2017):** Acute lymphoblastic leukemia: a comprehensive review and 2017 update. Blood Cancer Journal; 7 (e577).
13. **Margolin JF, Rabin KR, Steuber CP, and Poplack DG.** Acute lymphocytic leukemia. In: Pizzo PA and Poplack DG. Principles and practice of pediatric oncology; 6th ed. Philadelphia: JB Lippincott, Williams &Wilkins; 2011;19 (518)
14. **Vaitkeviciene G, Forestier E, Hellebostad M, Heyman M, Jonsson OG, Lahteenmaki PM, et al.** High white blood cell count at diagnosis of childhood acute lymphoblastic leukaemia: biological background and prognostic impact. Results from the NOPHO ALL-92 and ALL-2000 studies. European Journal of Haematology. 2010; 86(38).
15. **Chiaretti S, Vitale A, Cazzaniga G, Orlando SM, Silvestri D, Fazi P, et al.** Clinico-biological features of 5202 patients with acute lymphoblastic leukemia enrolled in the Italian AIEOP and GIMEMA protocols and stratified in age cohorts. Haematologica. 2013; 98(11),1702.
16. **Girardi T, Vicente C, Cools J, and De Keersmaecker K.** The genetics and molecular biology of T-ALL. Blood. **2017**;129(9),1113.
17. **Chao MP, Alizadeh AA, Tang C, Jan M, Weissman-Tsukamoto R, Zhao F, et al.** Therapeutic antibody targeting of CD47 eliminates human acute lymphoblastic leukemia. Cancer research. 2011; 71(4):1374-84.
18. **Tonks A, Hills R, White P, Rosie B, Mills KI, Burnett AK, Darley RL.**CD200 as a prognostic factor in acute myeloid leukaemia. Blood.2007; 109(5): 2015-2021.
19. **Arai,H., Gandhi,N., Battaglin,F., Wang,J. Algaze,S., Jayachandran,P. et al.** Role

of CD47 gene expression in colorectal cancer: a comprehensive molecular profiling study. Arai, et al. J Immunother Cancer. 2024; 12: e010326.

20. **Majeti R, Chao MP, Alizadeh AA, Pang WW, Jaiswal, S, and Weissman, IL.** CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. Cell.2009; 138(2), 286-299.
21. **Khalil M, Elsharkawy N, Mohsen M, Elmawardy MM, and Ayoub AM.** Prognostic role of CD200 in acute lymphoblastic leukemia patients. Diagnostics. 2024;(13),325.

الملخص العربي

دور منظم المناعه س د200 وس د 47 فى سرطان الدم الليمفاوى الحاد فى مرحلة الطفولة

إسراء عادل عياد[1]، ريهام محمود حماد[1]، رندة أمين عثمان [2]، منى حلمى الريس[1]

[1] قسم الباثولوجيا الإكلينيكية، كلية طب بنات، القاهرة ، جامعة الأزهر، جمهورية مصر العربية.

[2] قسم الباثولوجيا الإكلينيكية، المعهد القومي للأورام، القاهرة، جامعة القاهرة،، جمهورية مصر العربية.

ملخص البحث:

الخلفية: يُمثل سرطان الدم الليمفاوي الحاد حوالي 30% من جميع سرطانات الأطفال.وقد تُسهم قدرة خلايا سرطان الدم على تجنب تدمير الجهاز المناعي في ظهور المرض وانتشاره. يُعد س د 47 أحد مُنظمات الاستجابة المناعية التكيفية. يتفاعل مستقبل س د 200 ر، المُثبط لعائلة الغلوبولين المناعي، والذي يُعبَّر عنه غالبًا في خلايا سلالة النخاع/الوحيدات ومجموعة فرعية من الخلايا التائية، مع س د 200 ر. يُثبِّط مع س د 200 ر الاستجابات المناعية التي تُحرّكها الخلايا التائية والوحيدات. ويُعرف وجود خلايا سرطان الدم بعد العلاج في نخاع العظم باسم الحد الأدنى من المرض المتبقي (م ر د)

الهدف: تسليط الضوء على دور س د 200 و س د 47 في سرطان الدم الليمفاوي الحاد لدى الأطفال، مما قد يُساعد في فهم الأدوار التي يلعبانها، وإبراز دورهما المُحتمل في الحد الأدنى من المرض المتبقي.

الطرق: تم إجراء هذه الدراسة على 52 حالة من حالات سرطان الدم الليمفاوي الحاد لدى الأطفال من نوعي الخلايا البائية والتائية. تم تقييم نمط التعبير عن س د 200 و س د 47 على الخلايا الأرومية باستخدام قياس التدفق الخلوي. تم الكشف عن الحد الأدنى للقيمة (م ر د) في اليوم 42، الحد الأدنى لقيمة (م ر د) هو 0.01%.

النتائج: ارتفع التعبير عن س د 200 بشكل ملحوظ في مجموعة سرطان الدم الليمفاوي البائي الحاد مقارنةً بمجموعة سرطان الدم الليمفاوي التائي الحاد. كانت هناك زيادة ذات دلالة إحصائية في تضخم الكبد، وتضخم الطحال، وتضخم العقد اللمفاوية حول الأبهر، وكتلة المنصف الأمامية في مجموعة سرطان الدم الليمفاوي التائي الحاد مقارنةً بمجموعة سرطان الدم الليمفاوي البائي الحاد.

الاستنتاجات: ظهرت حالات سرطان الدم الليمفاوي الحاد التائي بمسار أكثر حده. ظهرت لدى مرضى سرطان الدم الليمفاوي البائي الحاد في البداية زيادة كبيرة في التعبير عن س د 200 على الخلايا الأرومية. لم يظهر أي فرق كبير في مستويات الحد الأدنى للجرعة في اليوم 42 بين مرضى سرطان الدم الليمفاوي البائي الحاد ومرضى سرطان الدم الليمفاوي التائي الحاد.

الكلمات المفتاحيه: س د47؛ س د200؛ سرطان الدم الليمفاوي الحاد.

الباحث الرئيسي:

الاسم : إسراء عادل عياد، قسم الباثولوجيا الإكلينيكية، كلية طب بنات، القاهرة ، جامعة الأزهر، جمهورية مصر العربية.

الهاتف: 0124559978

البريد الالكتروني: Esraaayad920@yahoo.com