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Original Article

Higher baseline natural killer cell counts are associated with a lower 8-day blast count and lower day 33 minimal residual disease in children with pediatric B-acute lymphoblastic leukemia

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Abstract: Introduction: Acute lymphoblastic leukemia (ALL) is the most common malignancy in children. Despite advancements in treatment, a significant proportion of children relapse. Recently, immunotherapy has gained momentum and is becoming popular, especially for relapsed and refractory cases. NK cells are an important part of tumor immunity and are involved in the direct killing of tumor cells. Their role in B-ALL has not been explored. Therefore, this study was conducted to correlate the number of NK cells with standard prognostic parameters in B-ALL. Methods: 25 subjects with newly diagnosed B-ALL between 0-14 years were recruited for the study from Pediatric OPD or emergency of the hospital. Along with a complete hemogram and peripheral smear examination, immunophenotyping by flow cytometry was done at the time of diagnosis for NK cell enumeration. The number of NK cells was correlated with standard prognostic parameters using the spearman correlation coefficient. Results: Baseline NK cell percentage demonstrated a significant negative correlation with Prednisone poor day 8 blast response (*P* value = 0.02, r value = -0.44) and positive MRD (*P* value = 0.01, r value = -0.49) at day 33. A negative correlation was also noticed between NK cell percentage and unfavorable cytogenetics (hypodiploidy), although it was not significant (*P* value = 0.06, r value = -0.38). The number of NK cells did not correlate with age, gender and WBC count. Therefore, evaluating NK cells at diagnosis may serve as a simple and useful parameter for prognostication and risk stratification. Conclusion: It may be assumed that a higher percentage of NK cells is associated with improved outcomes and probably a better prognosis. NK numbers may serve as an early independent parameter predicting prognosis and survival in children with B-ALL, thus helping to decide individual therapeutic regimens.

Keywords: B-ALL, NK cells, pediatric, prognosis, flow cytometry

Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children, accounting for 25% of all childhood cancers and approximately 75% of childhood leukemia [1, 2]. In India, nearly 25,000 children are diagnosed with ALL annually [3]. Amongst ALL, B cell ALL is much more common than the one with T cell origin. With the advent of modern chemotherapy and radiotherapy, cure rates in children have improved drastically to overall event-free survival of approximately 80% [2, 4]. Despite advancements, around 20 to 30% of children with ALL in whom remission is achieved after initial induction chemotherapy, will still relapse [5].

Multiagent chemotherapy used in these patients is mainly based upon prognostic parameters and risk assessment and tailored accordingly for individual patients. The standard prognostic parameters taken into account include patient age, baseline white blood cell (WBC) count, cytogenetic abnormalities (presence/absence of t(9,22) or t(4,11) chromosome abnormalities), and markers of early treatment response [6].

The current focus of therapy in children with ALL is reducing relapse rates and long-term side effects. Recently, immunotherapy has gained momentum and is becoming popular, especially for relapsed and refractory cases. Moreover, much focus has shifted to harnessing the vari-

ous components of the host immune milieu to assist the clearance of tumor cells from the body. Characterization of the cellular micro milieu in leukemic bone marrow (BM) may lead to a better understanding of the anti-leukemic host responses and provide a basis for developing immune-based strategies.

Cells of both adaptive and innate immune systems, CD8+ T cells, Th1-polarized CD4+ T cells, T regulatory cells, Natural killer (NK) cells, dendritic cells and macrophages are involved in the clearance of tumor cells from the body [7]. NK cells are part of both adaptive and innate immunity. They are involved in the direct killing of tumor cells by contact-dependent cytotoxicity and cytokine production for immune modulation [5, 8-10]. Target cell apoptosis is primarily mediated by Perforin and Granzyme B mediated pathways and the regulation of immune responses by the secretion of cytokines such as Interferon-γ and Tumor necrosis factor-α [11]. It can be inferred that the number of NK cells should correlate with a better tumor outcome. Not many studies have been conducted in patients with ALL regarding the role of NK cells. Milejska et al. in their study on pediatric ALL (both T and B), had shown that the number of NK cells in the bone marrow of children with acute leukemia at diagnosis was positively associated with a better outcome [12]. In a study on T ALL, Boieri et al. found a significantly lower proliferation of leukemic cells in the presence of NK cells, especially activated by IL-12, IL-15, and IL-18 [13]. Further, NK cell cytotoxic effects have been variably studied in various other malignancies, including breast cancer, Hodgkin's disease (HD), non-Hodgkin's lymphoma (NHL), multiple myeloma, solid tumors like head and neck, ovarian cancer, thyroid cancer, and melanoma [14]. It was also seen that breast cancer patients of all clinical stages of disease had significantly decreased NK cell activity as compared to healthy controls, and this was profound in the advanced stage of this disease [15, 16].

B-ALL is one of the most common pediatric tumors, but the prognostic implications of baseline NK cell counts in children with B-ALL have not been studied. Knowledge of how NK cells may influence tumor growth and response to therapy may open new therapeutic avenues involving manipulations in these cells to achieve better treatment outcomes. Therefore, this study was conducted to correlate the number of NK cells with standard prognostic parameters defined by the Acute Lymphoblastic Leukemia Intercontinental 2002 (ALL-IC 2002) treatment protocol to determine their prognostic significance [6].

Methods

Study design

This prospective cross-sectional study was conducted in the Department of Pediatrics and Pathology in a 1500 bedded tertiary care hospital in Delhi, India, over a fifteen months period (November 2019 to April 2021). Approval for the study was obtained from Institutional Ethics Committee for Human Research (IEC-HR) (IEC-HR/2019/41/119R). Written informed consent was taken from the parents/guardians of the study participants, and verbal assent was obtained as applicable for participants aged ≥ 7 years.

Study subjects

25 subjects between 0-14 years were recruited for the study from Pediatric OPD or emergency of the hospital. Inclusion criteria were newly diagnosed cases of B-ALL based on presence of > 20% blasts in peripheral blood or bone marrow belonging to B lineage confirmed by flow cytometric immunophenotyping and consenting to be a part of the study. The following subjects were excluded - Subjects with B-ALL already on chemotherapy and/or cytotoxic drugs; relapsed cases of B-ALL (defined by Centre for International Blood and Marrow Transplant Research as recurrence of disease after achieving complete remission, meeting at least one of the following criteria - ≥ 5% blasts in the marrow/peripheral blood or extramedullary disease or disease presence determined by physician upon clinical assessment); those not given BFM protocol 2002 as standard therapy; those who were non-compliant to treatment; subjects with known primary or secondary immunodeficiency disorder.

Sample collection and analysis

Complete clinical and demographic details were collected from case record sheets for every subject. At the time of presentation, 3 ml peripheral venous blood sample was drawn under aseptic conditions in an EDTA vial, out of

Table 1. Flow panel used for leukemia immunophenotyping

Fluorochrome	FITC	PE	ECD	PC5	PC7
TUBE 1	-	CD34	-	CD117	CD45
TUBE 2	CD13	CD33	HLADR	-	CD45
TUBE 3	CD19	CD10	CD45	CD20	CD22
TUBE 4	-	CD5	CD45	CD3	CD7
TUBE 5	cMPO	cCD79a	-	cCD3	CD45

which 1 ml was used for hemogram and peripheral blood smear examination and 2 ml for immuno-phenotyping by flow-cytometry. MRD was conducted on bone marrow aspirate collected on Day 33 of treatment as a part of standard protocol.

Hemogram: Hemogram was performed using a six-part automated hematology analyzer (Mindray BC-6800). Hemoglobin (Hb), hematocrit (Hct), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total leucocyte count (TLC) and platelet count was recorded for all subjects. Peripheral smear examination using Wright's stain was done at the time of diagnosis and on the 8th day of the start of Prednisolone therapy and the number of blasts was noted on both days.

Flow cytometry-based immuno-phenotyping for diagnosis, Minimal residual disease (MRD) assessment and NK cell enumeration: Immuno-phenotyping by flow cytometry was done at the time of diagnosis for B lineage confirmation as well as NK cell enumeration and was repeated as a part of standard protocol on the 33rd day for assessing MRD. It was done using a two-laser 5-color flow cytometer (FC-500 by Beckman Coulter Ltd). For Immuno-phenotyping, the stain-lyse-wash method was used according to conventional protocol. The sample was acquired on a Beckman Coulter FC-500 flow cytometer using Cytometry List Mode data (LMD) acquisition and analysis software (CXP Analysis 2.2). The quality control of the machine was done daily, and a 'Flow Check' was run every day to check for the machine settings.

B-ALL diagnosis

For diagnosis of B-ALL, 5 tubes were used with antibodies as shown in **Table 1**. Viability gating was done using SSC vs. FSC to exclude the debris. After putting the viability gate, blasts were gated as CD45 negative/dim and low side scatter characteristics. According to the WHO classification of Tumors of Hematopoietic and Lymphoid Tumors, diagnosis of B lineage ALL was made if there was the presence of strong CD19 and at least 1 of the following strongly expressed - CD79a, cCD22, CD10 OR weak CD19 with at least 2 of the following strongly expressed - CD79a, cCD22, CD10.

NK cell enumeration

For NK cell enumeration, a single tube flow-cytometric evaluation was performed using the antibodies shown in **Table 2**. Viability gating was done using SSC vs. FSC to exclude the debris. After putting viability gate, lymphocytes were gated based on bright CD45 and low side scatter characteristics. Further, CD4 and CD8 T-lymphocytes were identified by CD4 vs. CD8 dot plots applied to the CD3 gated population. NK cells were gated on CD3 vs. CD56 as CD3-ve and CD56+ve cells. Simultaneously NK like T cells was gated as CD3+ve, CD56-ve cells as shown in **Figure 1**. The relative percentages of all the cells were seen and recorded. Also, the CD4 to CD8 ratio was calculated.

Assessment of minimal residual disease

Minimal Residual Disease was studied on Day 33 of treatment for evaluation of Leukemia Associated Immunophenotype (LAIP) using following markers - CD19, CD20, CD10, CD34, CD45, CD58, CD200, CD38, CD123, and CD86. Target of 10 lac events were kept and a sample below 5 lac events was considered inadequate. MRD was defined as post remission persistence of > 0.01% leukemic cells [17].

Conventional cytogenetics was not available in-house. So, it was sent outside where it was done using the high-resolution G banding technique. The results were noted for all 25 patients.

Statistical analysis

The data collected was entered into a computer-based spreadsheet using MS Excel and cleaned. The cleaned data were analyzed using SPSS 20.0 (Statistical Package for Social Sciences version 20.0). The statistical analysis comprised calculating means and proportions.

Table 2. Flow panel used for evaluation of NK cells

TUBE 1	CD45	CD4	CD8	CD3	CD56
	J33	13B8.2	B9.11	UCHT1	N901
	(IgG1 Mouse)	(IgG1 Mouse)	(IgG1 Mouse)	(IgG1 Mouse)	(IgG1 Mouse)

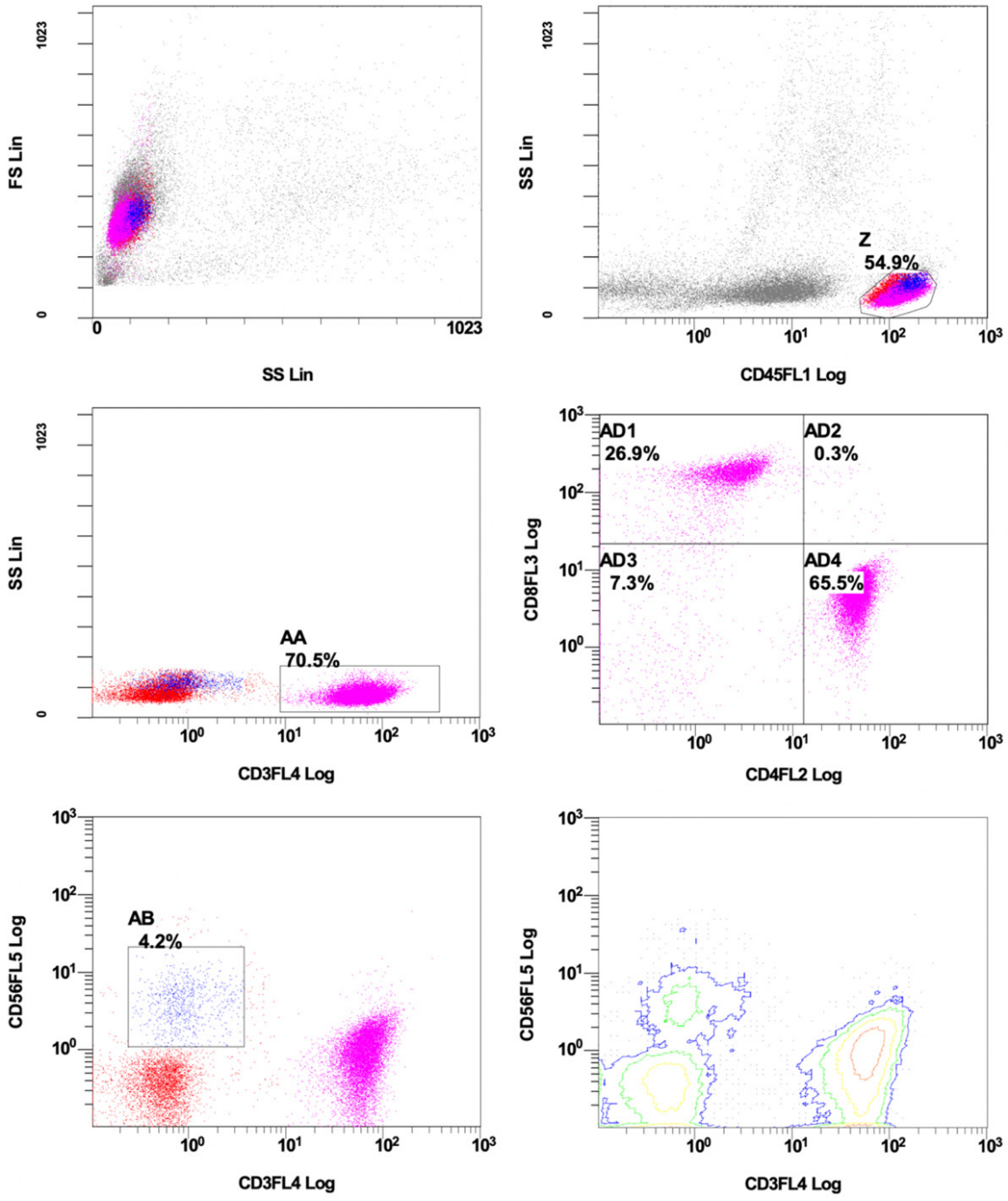

Figure 1. The figure shows gating technique used for NK cell enumeration by flow cytometry in study subjects. The dot plots and contour plots have been taken from one of the subject in the study.

Standard deviation or range was computed for the continuous variables to compute the dispersion within the variables. Correlation between the various prognostic factors was ana-

Table 3. Hematological parameters in study subjects (n = 25)

	At the time of diagnosis	Day-8th
	Mean ± SD	Mean ± SD
Hb (g/dL)	6.6 ± 2.09	7.124 ± 1.75
RBC (× 10^{12}/L)	2.4 ± 0.72	2.62 ± 0.60
TLC (× 10^9/L)	33.5 ± 3.624	4.6 ± 2.64
Platelet count (× 10^9/L)	42.4 ± 3.68	45 ± 18.5
Peripheral Blast count (%)	61.2 ± 20.11	2.22 ± 5

Table 4. Percentage of T cell populations and NK cell at diagnosis in study subjects (n = 25)

	Mean ± SD	Range
CD3% (of total lymphocyte)	74.29 ± 12.7	54.6-92.5
CD4% (of total lymphocyte)	41.44 ± 14.5	0.2-18.55
CD8% (of total lymphocyte)	45.9 ± 14.4	22.5-78.3
CD4/CD8 ratio	1.03 ± 0.60	0.004-2.37
NK cell % (of total lymphocyte)	4.24 ± 3.7	0.2-13.4

Table 5. Correlation of NK cell with Day 8 blast response, Day 33 MRD and cytogenetics

	r value	*P* value
Day 8 blast count	-0.442	0.027*
MRD day 33	0.494	0.012*
Cytogenetics	0.38	0.06

*denotes correlation is significant at 0.05 level, r-Correlation co-efficient. Statistical method: Spearman's rho correlation coefficient.

lyzed using spearman's correlation. The cut-off for defining statistical significance was taken as *P*-value < 0.05.

Results

The present study correlated NK cell counts with known prognostic parameters in children with B-ALL. The study included 25 subjects, 19 males and 6 females. The age of the subjects ranged from 1-10 years with a Mean ± SD of 5.80 ± 2.85. Of the 25 cases, 15 were from zero to six years, and 10 were between seven to ten years.

A complete evaluation of the hematologic profile of all patients was done at the time of diagnosis and on day 8. **Table 3** outlines the hematological details of the subjects. A significant drop in TLC on Day 8 was noted as expected. Peripheral blood blast count ranged from 20-90%, with a mean of 61% at diagnosis and a mean of 2.22% on day 8.

Prednisone response on Day 8 was determined by absolute blast count in peripheral blood on Day 8, after 7 days of prednisone and one dose of intrathecal methotrexate (MTX). According to ALL IC-BFM 2002, Prednisone poor response (PPR) was defined as blasts equal to or more than 1 × 10^9/L in peripheral blood. Prednisone good response (PGR) was defined as less than 1 × 10^9/L blasts in peripheral blood on Day 8. So, the patients with blasts > 1 × 10^9/L were considered positive for day 8 blasts or had a poor prednisolone response [18].

Out of 25 subjects in our study, 5 (20%) were positive for blast on day 8 of treatment with steroid chemotherapy and thus showed a PPR. Further, 11 out of 25 (44%) patients were MRD positive (> 0.01% leukemic cells in the marrow) on Day 33. Conventional cytogenetics was done in all 25 patients using high-resolution G banding. 4 out of 25 (16%) patients showed unfavorable cytogenetics - all four had hypodiploidy.

Percentage of NK cells and correlation

NK cell % in cases ranged from 0.2-13.4 with a Mean ± SD of 4.24 ± 3.7. **Table 4** shows the percentage of different T cell populations and NK cells at diagnosis. The number of NK cells was correlated with other prognostic parameters. Baseline NK cell percentage demonstrated a significant negative correlation with Prednisone poor day 8 blast response and positive MRD at day 33 (**Table 5**). A negative correlation was also noticed with unfavorable cytogenetics (hypodiploidy), although it was not significant. The number of NK cells did not show any correlation with age, gender and WBC count.

Discussion

This prospective cross-sectional study was conducted in a tertiary care hospital over fifteen months and included 25 newly diagnosed pediatric B-ALL children. This study was done to find the correlation of NK cells with standard prognostic parameters in B-ALL patients to understand the role of NK cells in prognosis.

NK cell percentage was enumerated using CD56 and CD3. CD3-ve/CD56+ve cells were counted as NK cells, and their percentage (out of the total lymphocytes) was noted. The presence of NK cells demonstrated a significant negative correlation with prednisone poor day 8 blast response (*P*-value < 0.02) and MRD positivity at day 33 (*P* value < 0.01).

Our results were in concordance with some of the studies conducted on NK cells. In a study conducted in Poland between 2005 and 2013 by Agnieszka and Milejska et al. on 84 children with ALL (both B and T cell), NK cell percentage in bone marrow was assessed using flow cytometry and correlated with prognosis and overall survival. They divided the patients into two groups based on NK cell percentage - a group I (NK+) patients with NK cells (> 1%) in the bone marrow and group II (NK-) patients without NK cells (< 1%) in the bone marrow. Patients in group I had a significantly higher frequency of Prednisone response on day 8 and marrow remission on day 15 of treatment (*P* = 0.01, *P* = 0.03). Further, children from group I had significantly better survival than those from group II (*P* = 02). Thus, the study demonstrated a significant and direct correlation between good prednisone response on day 8 and the remission on day 15 of treatment with a percentage of NK cells in the bone marrow [12]. Another study conducted by Boieri et al. on patients with T-ALL showed significantly lower proliferation of leukemic cells in T cell. Acute lymphoblastic leukemia in the presence of NK cells, especially activated by IL-12, IL-15, and IL-18. The authors contemplated that this could be used to target blast and have potential implications for immunotherapeutic protocols using NK cells to more efficiently target leukemia [13].

Despite an extensive search of the literature, we could not find any research studying the role of NK cells in pediatric B-ALL. Research was conducted by Jamal et al. on patients with AML to investigate the clinical impact of the percentage of Natural Killer Cells and Natural Killer like T cell populations in these patients. They collected the bone marrow and peripheral blood samples from 50 adult patients who presented to the Hematology Unit in the Oncology Center Mansoura University (OCMU) at the time of diagnosis. NK and NK-like T cells were detected using immuno-phenotyping by expression of cell surface and cytoplasmic markers. They observed a significant reduction in the median values of NK and NK T cells in AML patients compared to normal values [19].

Similarly, studies on the number of NK cells and their cytotoxic effect have been conducted on various other malignancies, including breast cancer, Hodgkin's disease (HD), non-Hodgkin's lymphoma (NHL), multiple myeloma, in solid tumors like head and neck, ovarian cancer, thyroid cancer, and melanoma [14, 16]. A study by Konjevik et al. demonstrated that breast cancer patients of all clinical stages of disease had significantly decreased NK cell activity compared to healthy controls [15]. A study done by Tang et al. on colorectal cancer patients demonstrated that the percentage of NK cells in the blood was an independent predictor of survival [20].

Overall, the study found that:

- Despite standard chemotherapy, a significant percentage of children with B-ALL relapse. This further leads to intensification of chemotherapy and thus increase in side effects as well as risk of infection and need for blood products. Further, many setups are not well equipped with management of such children making their treatment difficult. Thus, it is time to explore newer avenues with lower toxicity and better patient compliance.

- NK cell percentage showed a negative correlation with Prednisone poor response at 8th day and MRD at 33 days of therapy. Thus, it can be said that a higher percentage of NK cells is associated with improved outcomes and probably a better prognosis. This can be explained by the fact that NK cells have tumor cytotoxic effect by the production of powerful target cell-induced cytokine and cytotoxicity [21]. Exploring cytotoxic properties of NK cells may open newer avenues for immunotherapy. Further, NK numbers may serve as an early independent parameter predicting prognosis and survival in children with B-ALL, thus helping to decide individual therapeutic regimens.

In this study, no significant correlation was found between NK cell numbers and WBC count, age, gender, cytogenetics. This needs to be further evaluated as the present study had a small sample size. This was the main limita-

tion of the study. More studies in future on NK cells with larger sample sizes along with follow-up at different stages are needed to further validate these preliminary findings.

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Disclosure of conflict of interest

None.

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References

[1] Pui CH and Evans WE. Acute lymphoblastic leukemia. N Engl J Med 1998; 339: 605-615.

[2] Gaynon PS. Childhood acute lymphoblastic leukemia and relapse. Br J Haematol 2005; 131: 579-587.

[3] Arora RS and Arora B. Acute leukemia in children: a review of the current Indian data. South Asian J Cancer 2016; 5: 155-160.

[4] Shanta V, Maitreyan V, Sagar TG, Gajalakshmi CK and Rajalekshmy KR. Prognostic variables and survival in pediatric acute lymphoblastic leukemias: cancer institute experience. Pediatr Hematol Oncol 1996; 13: 205-216.

[5] Torelli GF, Peragine N, Raponi S, Pagliara D, De Propris MS, Vitale A, Bertaina A, Barberi W, Moretta L, Basso G, Santoni A, Guarini A, Locatelli F and Foà R. Recognition of adult and pediatric acute lymphoblastic leukemia blasts by natural killer cells. Haematologica 2014; 99: 1248-1254.

[6] Stary J, Zimmermann M, Campbell M, Castillo L, Dibar E, Donska S, Gonzalez A, Izraeli S, Janic D, Jazbec J, Konja J, Kaiserova E, Kowalczyk J, Kovacs G, Li CK, Magyarosy E, Popa A, Stark B, Jabali Y, Trka J, Hrusak O, Riehm H, Masera G and Schrappe M. Intensive chemotherapy for childhood acute lymphoblastic leukemia: results of the randomized intercontinental trial ALL IC-BFM 2002. J Clin Oncol 2014; 32: 174-184.

[7] Johansson M, Denardo DG and Coussens LM. Polarized immune responses differentially regulate cancer development. Immunol Rev 2008; 222: 145-154.

[8] Koltan S, Debski R, Koltan A, Grzesk E, Tejza B, Eljaszewicz A, Gackowska L, Kubicka M, Kolodziej B, Kurylo-Rafinska B, Kubiszewska I, Wiese M, Januszewska M, Michalkiewicz J, Wysocki M, Styczynski J and Grzesk G. Phenotype of NK cells determined on the basis of selected immunological parameters in children treated due to acute lymphoblastic leukemia. Medicine (Baltimore) 2015; 94: e2369.

[9] Georgeson GD, Szony BJ, Streitman K, Kovács A, Kovács L and László A. Natural killer cell cytotoxicity is deficient in newborns with sepsis and recurrent infections. Eur J Pediatr 2001; 160: 478-482.

[10] Ogata K, An E, Shioi Y, Nakamura K, Luo S, Yokose N, Minami S and Dan K. Association between natural killer cell activity and infection in immunologically normal elderly people. Clin Exp Immunol 2001; 124: 392-397.

[11] Chowdhury D and Lieberman J. Death by a thousand cuts: granzyme pathways of programmed cell death. Annu Rev Immunol 2008; 26: 389-420.

[12] Mizia-Malarz A and Sobol-Milejska G. NK cells as possible prognostic factor in childhood acute lymphoblastic leukemia. Dis Markers 2019; 2019: 3596983.

[13] Boieri M, Ulvmoen A, Sudworth A, Lendrem C, Collin M, Dickinson AM, Kveberg L and Inngjerdingen M. IL-12, IL-15, and IL-18 preactivated NK cells target resistant T cell acute lymphoblastic leukemia and delay leukemia development in vivo. Oncoimmunology 2017; 6: e1274478.

[14] Appelbaum JW. The role of the immune system in the pathogenesis of cancer. Semin Oncol Nurs 1992; 8: 51-62.

[15] Konjević G and Spuzić I. Stage dependence of NK cell activity and its modulation by interleukin 2 in patients with breast cancer. Neoplasma 1993; 40: 81-85.

[16] Spuzić I and Konjević G. The significance of determination of NK cell activity in malignant diseases. Glas Srp Akad Nauka Med 1990; 39: 153-160.

[17] Borowitz MJ, Devidas M, Hunger SP, Bowman WP, Carroll AJ, Carroll WL, Linda S, Martin PL, Pullen DJ, Viswanatha D, Willman CL, Winick N and Camitta BM; Children's Oncology Group. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. Blood 2008; 111: 5477-585.

[18] Möricke A, Zimmermann M, Reiter A, Henze G, Schrauder A, Gadner H, Ludwig WD, Ritter J, Harbott J, Mann G, Klingebiel T, Zintl F, Niemeyer C, Kremens B, Niggli F, Niethammer D, Welte K, Stanulla M, Odenwald E, Riehm H and Schrappe M. Long-term results of five con-

secutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000. Leukemia 2010; 24: 265-284.

[19] Jamal E, Azmy E, Ayed M, Aref S and Eisa N. Clinical impact of percentage of natural killer cells and natural killer-like T cell population in acute myeloid leukemia. J Hematol 2020; 9: 62-70.

[20] Tang YP, Xie MZ, Li KZ, Li JL, Cai ZM and Hu BL. Prognostic value of peripheral blood natural killer cells in colorectal cancer. BMC Gastroenterol 2020; 20: 31.

[21] Metelitsa LS, Naidenko OV, Kant A, Wu HW, Loza MJ, Perussia B, Kronenberg M and Seeger RC. Human NKT cells mediate antitumor cytotoxicity directly by recognizing target cell CD1d with bound ligand or indirectly by producing IL-2 to activate NK cells. J Immunol 2001; 167: 3114-3122.