Chronic Diseases Prevention Review 2 (2017) 18-26

Available at http:// www.cancercellresearch.org ISSN 2158-0820

Study of the levels changes of nk cells in different stages of chemotherapy in children with acute leukemia

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Abstract: **To detect the activity and quantity of NK cells in different stages of chemotherapy in children with acute leukem and to explore its significance of immunotherapy. NK cell was detected by flow cytometry with CD107a labeling antibody in peripheral blood from 16 cases of children with newly diagnosed acute leukemia and 46 cases of children with complete remission (CR). 15 cases of healthy children were used as normal control group. 46 cases of children with CR were divided into three groups according to duration of treatment, including 16 cases with CR last for 6-9 months (CR-A group), 14 cases last for 1-1.5 years (CR-B group), 16 cases with CR over 2 years (CR-C group). The quantity and activity of NK cells in newly diagnosed group were significantly lower than the control group (P<0.05). The quantity in CR group was (7.01±2.76)%, and was significantly higher than newly diagnosed patients (P<0.05). The quantity of NK cells in CR-A group, CR-B group, CR-C group was significantly higher than the newly diagnosed group (P<0.05), but there was no significant difference (P>0.05) between CR-A group and CR-B group. The CD 107a labeling antibody by flow cytometry and LDH release assay were positively correlated and the correlation coefficient was 0.754. CD107a labeling antibody by flow cytometry correlates with the LDH releaseassay positively, and its specific, simple, reproducibility detection method. The quantity and activity of newly diagnosed group were lower than normal controls, with time goes by, they were increased after CR 6-9 months and one year, but they are still lower than the normal even CR 2 years later.**

Keywords: Acute leukemia; NK cell activity; CDl07a; Flow cytometry; LDH release assay

Received 10 February 2017, Revised 23 April 2017, Accepted 25 April 2017

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1. Introduction

Acute leukemia is the most common malignant hematologic disorder in childhood. Currently chemotherapy combined with hematopoietic stem cells transplantation in children with acute leukemia (AL), the long-term survival rate was 60-80%, but there was still about 30% of children with the final relapses. Leukemia relapse resulted from residual leukemia that cannot be removed, so immunotherapy has showed its advantage which the conventional treatment cannot be replaced for removing residual leukemia, increasing prognosis of AL. In recent years, the basic research of NK cells is hotter than before and it already gets some achievement, moreover, it contributes to the clinical application. It also provided a strong support to the development of immunotherapy of NK cells in children with acute leukemia. But what's NK cells function in the process of chemotherapy in children with acute leukemia and the process of reconstruction? That is an important basis for NK cell immunotherapy, and the assessment of prognosis in children acute leukemia. But by literature retrieval we found that in this area rarely reported at home and abroad. Some studies [1,2] have shown leukemia patients' NK cell activity after complete remission was significantly higher than the initial period, but the lack of different stages of chemotherapy in children with NK cell activity. Therefore, in this study, we used flow cytometry to detect the activity and quantity of NK cells at different stages of chemotherapy. In order to reveal the reconstruction of NK cells' immune function in children with leukemia, increase the immune status of children with leukemia and to provide basic information for immunotherapy of NK cells.

The detection method of NK cell activity is an important means, there are a variety of methods, but each have their own advantages and disadvantages. There are often unstable, poor repeatability, insensitive, isotope pollution concerns and time-consuming and other issues. But based on CD107a-labeled [3] detection of NK cell activity has the advantages of rapid, sensitive and less effector cells. In recent years, it was gradually applied to laboratory and clinical research of new detection methods. Nearly 2-3 years there were a few of this method reported [4], for detection of NK cell activity in patients with hemophagocytic lymphohistiocytosis. This study also combined with domestic and foreign customs which based on CD107a-labeled NK cell activity assay. It was used to establish the CD107a-labeled NK cell activity of leukemia methods.

2. Materials and Methods

2.1. Materials

2.1.1. Study subjects

From December 2013 to March 2014, 16 cases of children with newly diagnosed acute leukemia were enrolled in our hospital. 46 cases of complete remission, age and gender characteristics in Table 1, diagnosis and treatment of acute lymphoblastic leukemia in 2008 Year of childhood leukemia in China developed the CCLG-ALL2008 program, diagnosis and treatment of acute myeloid leukemia in 2006 Chinese Medical Association Pediatric Branch of the blood group to develop acute myeloid leukemia diagnosis and treatment of land and lead [5,6]. 15 healthy children as the normal control group, aged 0-12 years, the median age of 6 years (Table 1).

Table1 Clinical data of the subjects

Groups		n	Sex		Age (Y)	Median age (Y)
			Male	Female		
Newly diagnosed	ALL	11	6	5	0-10	8
	ANLL	5	2	3	1-12	
CR-A	ALL	14	7	7	3-11	5
	ANLL	2	2	0	1-13	
CR-B	ALL	12	7	5	3-9	7
	ANLL	2	1	1	7-14	
CR-C	ALL	13	5	8	3-14	6
	ANLL	3	3	0	8-13	
Control		15	7	6	1-12	6

2.1.2. Main experimental reagents

Gibco Fetal Bovine Serum (Yu Bo Biological (Shanghai) Technology Co.Ltd), RPMIl640 medium (Hyclone, USA), Lymphocyte separation solution (Tianjin Hao Yang biological products company), Anti-CDl07a-human-FITC (eBscience Inc., USA), Anti-CD3-human-PE (Beckman, USA), Anti-CD56-human-PC5 (Beckman, USA), K562 cell line is a gift from Professor Song of Medical College Cancer Hospital in Shandong.

2.2. Experimental methods

2.2.1. Cell culture and cryopreservation of human erythroleukemia K562

K562 cells were seeded in 10% inactivated fetal bovine serum10ml (1ml inactivated fetal bovine serum and 9ml containing 100U/ml penicillin and100U/ml gentamicin in sterile RPMIl640 culture medium) at 37 ℃, 5% $C0_2$ incubator culture, changing the fluid every 48h 1 to 1:2 ratio of subculture, inverted phase contrast microscope observed K562 cells. The well-conditioned logarithmic growth phase cells were used for the experimental study, and the remaining cells were frozen (10% DMS0 + 40% fetal bovine serum +50% RPMl1640) at room temperature 4 ℃ refrigerator (30min), refrigerator at 20 ℃ (30min) and -80 ℃ refrigerator (1h) followed by a cryogenic liquid nitrogen frozen.

2.2.2. CD107a-based detection of NK cell activity

Principle of the experiment: lysosome-associated membrane protein-1 (LAMP-1 or CD107a) is a membrane protein that accounts for about lysosomal 50% of the high glycosylated protein [7]. When NK cells kill the target cells, NK cells and the cell membrane fusion of toxic particles,

Causing the release of particulate matter will lead to the death of target cells. With the occurrence of NK cell degranulation, CD107a Molecules are transported to the cell membrane surface, while CD107a molecule expression and perforin secretion is consistent with the mouth of one. Because of this, NK cells expressing CD107a molecules may represent NK cells with cytotoxic activity.

Specimen collection and peripheral blood mononuclear cells (PBMCs) extraction:

(1) The peripheral blood 2ml (EDTA anticoagulant) was extracted and diluted with 2ml PBS.

(2) Take a sterile 15ml centrifuge tube, according to peripheral blood dilution: lymphocyte separation solution in 1: 1ratio, the peripheral blood dilution. The solution was slowly added to the supernatant of lymphocyte separation medium, centrifuged at 2500r/min at room temperature for 15min.

(3) Suction centrifugal tube in the white layer, adding sterile centrifuge tube, adding 5mlPBS solution, fully mixing, 1500r/min, Centrifuged 5min, washed twice.

(4) Discard the supernatant, by adding 10% inactivated fetal bovine serum RPMll640 medium, adjust the cell count 1×10^6/L, spare.

PBMCs were co-cultured with K562 cells:

K562 cells were used as target cells (adjusted cell

count 1×10^8/L). PBMCs were incubated with K562 Cells were mixed in 3:1 ratio (PBMCs cell solution 750ul, K562 cell solution 250ul) were inoculated in 24-well culture Plate, as the activation group (A), at 37 ℃, 5% $C0_2$ in an incubator for 2h, and then added to the medium Monensin sodium 2μmol/L, in the incubator for 1.5h. At the same time, PMBCs cell solution (750μl) Add 10% inactivated fetal bovine serum RPMI1640 medium 250μl, adjust the final volume 1ml, placed in 37 ℃, 5% $C0_2$ incubator, without target cells, cultured 3.5h, as resting group (R).

Flow cytometry analysis of NK cell surface CD107a expression:

The culture plates were removed from the incubator, and the activated and resting cells were transferred to 2ml of EP using a 1000ul. Tube, 1500r/min, 5min centrifugation, discard the supernatant, washed with PBS buffer 2 times, adjust the final volume of 100ul. And then anti-CD107a-human-FITC, anti-CD3 human-PE, Anti-CD56-human-PC5 each 5μl, while another EP tube plus FITC-IgG1 antibody (Beckman). As a control, at room temperature, dark staining 30min, after 1500r/min, 5min centrifugation, with PBS buffer Washed twice, discard the supernatant, add the sheath fluid (Coulter I soton Ⅱ Diluent) 500μl, upper machine flow cell CD107a expression on the surface of PMBCs and the number of NK cells were measured by CD3/CD56 Door, Cell Quest software analysis results.

2.2.3 LDH release of NK cell activity measured

At the beginning of the study, 15 cases of children with the detection method of CD107a-based markers and LDH release method to detect NK activity. LDH release method Experimental principle: LDH (lactate dehydrogenase) is a very stable cytoplasmic enzyme, presenting in the cytoplasm of normal cells. It cannot through the cell membrane, when the NK cells and target cells (K562 cells) were co-cultured. The target cells were damaged and the LDH was released into the extracellular space. The LDII could be abolished by the spleen. And INT (tetrazolium salts) reaction, the chapter into the red armor of the Shu-chi. It can be detected by microplate reader, collecting visible light 490nm wavelength absorbance data. It can determine the extent of cell damage. The experimental schematic diagram is as follows (Figure 1):

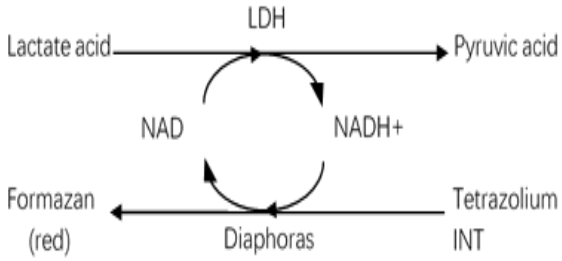

Figure 1. LDH release method Experimental principle.

(1) Subjects: 15 cases of children with LDH release method for simultaneous detection of NK cell activity.

(2) preparation of the kit:

a. INT solution (I*) configuration: Take the kit 10* INT solution 200μl, add 1800μl LINT dilution, configure 2 ml of the solution (1*).

b. LDH

Detection times	1	10	20	100
Lactic acid solution	20μl	200μl	400μl	2ml
INT solution(1*)	20μl	200μl	400μl	2ml
Enzyme solution	20μl	200μl	400μl	2ml
Total capacity	60μl	600μl	1200μl	6ml

(3) Steps:

① Extraction of OPBMCs, same as 2.2.1 collection of specimens.

② Cryopreservation of PBMCs, same as 1.2.2 K562 cells were cryopreserved.

③ RPMI-1640 medium containing 10% inactivated fetal bovine serum was used to adjust the target cells K562 cell density was 0.5×10^6ml and the effector cells were adjusted to a cell density of 1×10^6/ml of the cell suspension.

④ Grouping of each well was as follows: natural release pore of effector cell, natural release pore of target cell, target cell hole, target Cell release hole, each set of three complex holes (each group specific sample see Table 2).

Table 2 samples in Culture wells

Culture wells	K562 (μl)	PBMCs (μl)	Culture medium(μl)
spontaneously release of Effector cells	-	350	175
spontaneously release of Target cells	175	-	350
test group	175	350	-
The maximum release of target cells	175	-	350

*The culture medium was RPMI-1640 culture medium containing 10% inactivated fetal bovine serum, and the ratio was 10:1.

⑤ The 24-well plate was incubated for 4 hours in a 37℃, 5% $C0_2$ incubator, and 1 hour before the scheduled detection time. The LDH release reagent was added to the maximum release pore of the target cells in the amount of the original culture medium Of the 10%, mix, continue to culture in the incubator.

⑥ After the culture for 4 hours, the culture plate was taken out from the incubator, the cell suspension was transferred to the EP tube,1500r / min, 5min, centrifugation, respectively, the supernatant of each hole 120 "i (Note that set up three holes), added to the new 96 Orifice plate. Add the 60PL configured LDH detection working fluid into each well, mix and keep it at room temperature for 30min, then The absorbance was measured at 490 nm using a microplate reader.

2.3. Data processing

Detection of NK cell activity based on CD107a labeling Data processing: Final NK cell activity was calculated as follows NK cell activity (%) = CD107a positive rate (%)-stimulated CD107a expression (%).

LDH cell activity detection method Data processing: cell killing rate (%) = [(experimental group released an effect of spontaneous cells Release of a target cell spontaneously) / (target cell maximum release of a target cell spontaneous release)] × 100%.

2.4. Statistical analysis

SPSSl7.0 software was used to analyze the experimental data. The results are presented as mean ± SD. Between the two groups using t test, Wilcoxon rank sum test, multiple groups using one-way ANOVA between the mean The LSD-t test was used for comparison. Spearman correlation analysis was used for correlation test, with P<0.05 for the difference statistically significant.

3. Results

3.1. K562 cell growth was observed

The morphology of K562 cells was semi-adherent suspension, round cell body, translucent cells, morphological regularity under inverted microscope (Figure 2).

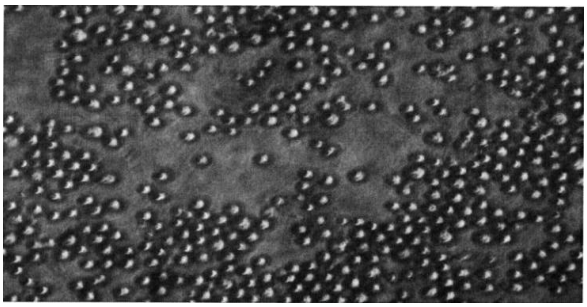

Figure 2. K562 cells in the logarithmic phase of growth (100*).

3.2. Comparison of NK cell activity assay with LDH release assay with CD107a-based

At the beginning of the study, 15 cases of children with CD107a-based markers and LDH release method to detect NK activity, the correlation analysis showed that, based on CD107a labeled NK cells activity detection method and LDH release method was positively correlated, R = 0.754, P <0. 01 (Table 3).

Table 3 Comparison of two detection methods (X±S)

Detection method	NK Cell viability(%)	R
CD107a-based Markers	11.57±3.70	0.754
LDH release	16.30±4.23	

3.3 The quantity of NK cells of Newly diagnosed patient with acute leukemia

3.3.1. Newly diagnosed patient with ALL and ANLL the number of NK cells were compared

Wilcoxon rank sum test was used to two independent samples, ALL group Rank sum T = 99, n=11, ANLL group Rank sum T = 37, n= 5.95% Bilateral probability limit is 24-61, and P<0.05. The results were as follows: under the test levels α= 0.05, ALL and ANLL the quantity of NK cells in different distribution (Table 4).

Table 4 NK cells between ALL and ANLL of newly diagnosed group

Groups	n	M(%)	P25-P75(%)
ALL	11	6.52	3.30-7.39
ANLL	5	5.61	1.67-11.25

3.3.2. Newly diagnosed patient acute leukemia and normal control group, the number of NK cells

The percentage of CD3 + CD56 + cells in peripheral blood was determined by flow cytometry (FCM). The percentage of CD3 + CD56 + cells in the control group was (10.80± 2.01)% and (3.86 ± 2.14)%, respectively (Table 5). Compared with the control group, the incidence of new leukemia was significantly lower Normal control group (P <0.05), the difference was statistically significant.

Table 5 The quantity of NK cells in peripheral blood of newly diagnosed acute leukemia and health children($\bar{X}$+s)

Groups	n	number of NK cell(%)	P
Control group	15	10.80±2.01	0.001
Newly diagnosed	16	3.86±2.14	

3.4. NK cell activity of newly diagnosed patient of leukemia

3.4.1. NK cell activity comparison between ALL and ANLL of newly diagnosed patient

Using the Wilcoxon rank sum test of two independent samples, ALL group rank sum T= 80, n = 11, ANLL group rank sum T = 56, n=5.95% bilateral probability limit is 24-61, so the P<0.05. The results showed: the distribution of NK cell activity of ALL and ANLL was not the same at α= 0.05 in both sides, and the ANLL was significantly lower than that of ALL Cell activity (Table 6).

Table 6 Activity of NK cells between ALL and ANLL of newly diagnosed group

Groups	n	M(%)	P25-P75(%)
ALL	11	5.30	2.80-7.80
ANLL	5	10.37	4.49-17.41

3.4.2 NK cell activity comparison between newly diagnosed acute leukemia and normal control group

CD107a-labeled NK cell activity assay was used to detect neonatal leukemia and normal children NK cell activity in the peripheral blood of the newly diagnosed group was (5.07±2.79)%, while in the normal control group (19.27±1.64)% (Table 7, flow diagram shown in Figure 3), compared with each other, the difference was statistically significant (P <0.05).

Table 7 The activity of NK cells in peripheral blood of newly diagnosed acute leukemia and health children (X+s)

groups	n	NK cell(%)	P
Control group	15	19.27+1.64	0.001
Newly diagnosed	16	5.07+2.79	

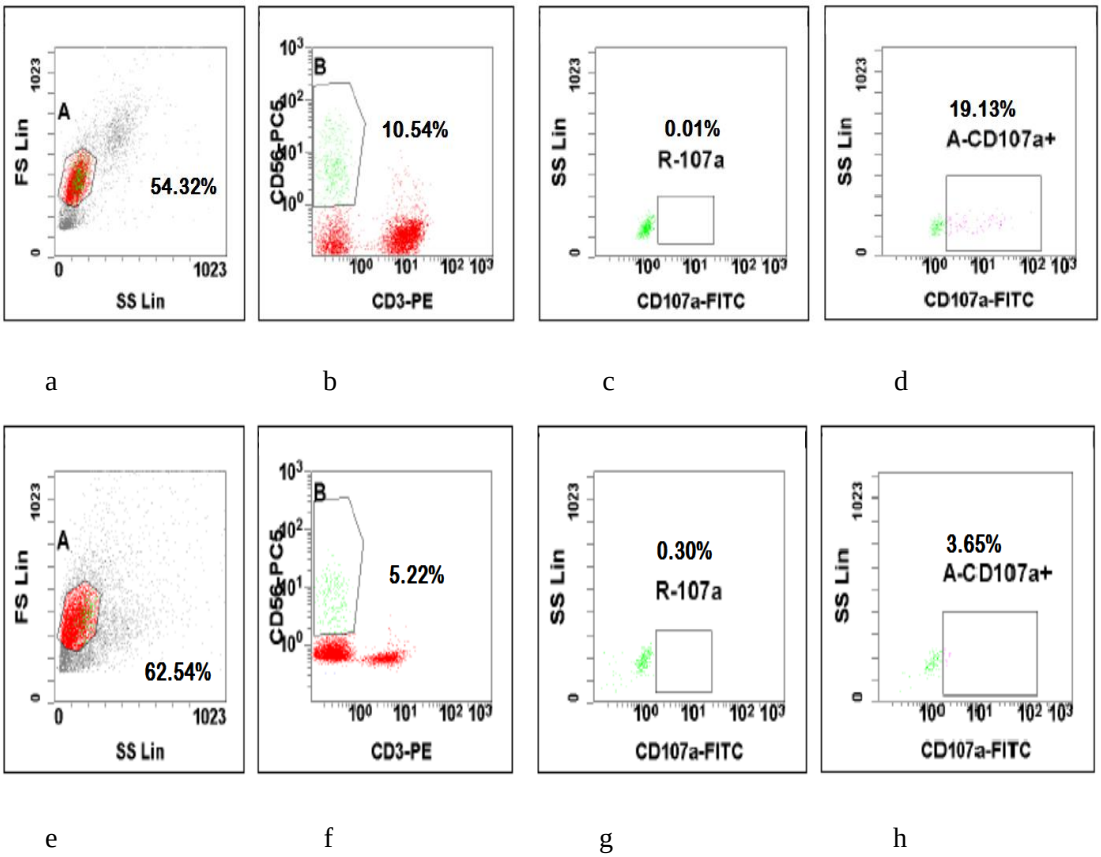

Figure 3. The activity of NK cells between normal control group and newly diagnosed group a-d refer to normal control group, e-h refers to newly diagnosed group.

2.5 Changes of NK cells in different stages of chemotherapy in children with leukemia

2.5.1 Comparison of the quantity of NK cells in different periods of chemotherapy treatment

The percentage of peripheral blood CD3+ and CD56+ cells in different treatment periods was detected by flow cytometry, the quantity of NK cells of newly diagnosed group was (3.86 ± 2.14)% and the CR-A group was (5.97±2.19)%, CR-B group was (6.59 ± 3.11)%, CR-C group was (8.36 ± 2.42)% (Table 8, Figure 4). There was no significant difference (P>0.05) between CR-A group and CR-B group, the rest comparisons between each other were significant difference (P <0.05).

Table 8 Comparison of NK cells in peripheral blood of acute leukemia patients at different stages of chemotherapy ($\bar{X}$+s)

groups	n	number of NK cells(%)	P			
Newly diagnosed	16	3.86±2.14	-	P1	P2	P3
CR-A	16	5.97±2.19	P1	-	P4	P5
CR-B	14	6.59±3.11	P2	P4	-	P6
CR-C	16	8.36±2.42	P3	P5	P6	-

P1 = 0. 025, newly diagnosed group compared with CR-A, P2 = 0.030, newly diagnosed group compared with CR-B
P3 = 0. 001, newly diagnosed group compared with CR-C, P4 = 0. 501, CR-A compared with CR-B
P5 = 0. 012, CR-A compared with CR-C, P6 = 0. 048, CR-B compared with CR-C

Table 9 Comparison of the quantity of NK cells in CR-C group and normal control group(X+s)

groups	n	Quantity of NK cells(%)	P
CR-C	16	8.36±2.42	0.009
Control group	15	10.80±2.01	

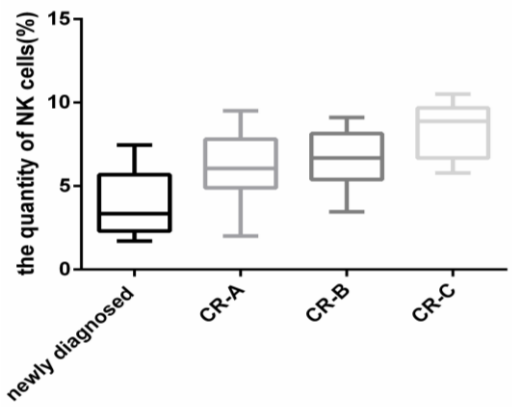

Figure 4. Comparison of the quantity of NK cells in different group.

2.5.2 Comparison of NK cells between CR-C group and normal control group

The quantity of CD3-CD56+ cells were detected by flow cytometry in CR-C group and normal control group, The quantity of NK cells was (8.364-2.42) % in CR-C, and (10.80+2.01)% in normal control group(Table 9), CR-C group was significantly lower than the normal control group (P<0.05).

4. Discussion

Acute leukemia is the most common malignant hematologic disorder in childhood. Currently combined with chemotherapy and hematopoietic stem cells transplantation of children with acute leukemia, the long-term survival rate was 60-80%, but there were still about 30% of children with the final relapse. Leukemia relapse resulted from residual leukemia can't be removed, so immunotherapy has showed its advantage which the conventional treatment cannot be replaced for removing residual leukemia, increasing prognosis of AL.

Immune surveillance of the body's plays an important role in removing the tumor. In the past, the secondary immune was the most important to tumor clearance, so immunotherapy research mostly around the T-lymphocyte. Nearly 10 years, the basic research of NK cells is very hot, and more and more progress has been made, and it was contributed to the clinical application of NK cells. Among this research, especially from the case of successful hematopoietic stem cell transplantation, we found that the activation of NK cell was related to prognosis of disease. It provided a strong support to immunotherapy.

NK cells accounted for about 15% of peripheral lymphocyte cycle, is an important part of the innate immune system. NK cells can be mediated activity and produced cytokines without prior sensitization to remove foreign matter, and differences normal cells in vivo. NK cells activation play an important role in killing, depending on the intracellular signal transduction pathways, depending on the balance between inhibitory and activating signals. According to the structural features of inhibitory receptors, NK cell surface expression of inhibitory receptors and activated receptors can be divided into immunoglobulin superfamily (Ig-SF) and C-Type lectin family (C-type lectin). The common feature of these inhibitory receptors is contained in the cytoplasm immunoreceptor tyrosine-based Inhibition Motif (ITIM). Immunoglobulin superfamily receptors, divided into killer cell immunoglobulin-like receptors (KIRs) and white cellular immunoglobulin-like receptors (LIRs), such

as KIR-2DL1, KIR-2DL2, KIR-2DL3, KIR-2DL4, KIR-2DL5A, KIR-2DL5B and KIR-3DLI, KIR-3DL2, KIR-3DL3, etc.; c-type lectin domain family into Members, such as CD94: NKG2, Ly49. Most of the ligands of inhibitory receptors are MHC class I molecules and abnormal cells (infected or tumor cells) expressing downregulated molecules. NK cells don't kill the normal cells that express autologous MHC-1 molecules. Activation Receptors include, in addition to some molecules that are structurally the same as inhibitory receptors (eg, lg-SF), including natural cytotoxicity (CD16, NKp44, NKp46, NKp80), membrane integrin molecules, receptors that recognize non-self antigen molecules (Ly49H) And other receptors (such as CD18, CD2, TLR-3, TLR-9) and so on. Activated receptor ligand is a non-molecular, such as ectopic expression of viral proteins and abnormal cellular expression of up-regulated molecules. NK cells surface can simultaneously express KAR (activated receptor) and KIR (inhibitory receptor), they play a synergistic biological effects. Antonio Pemz-Martnez [9] reported that AML patients receiving HLA haploidentical hematopoietic stem cell transplantation, when the donor NK cells inhibitory receptor KIR and recipients of MHC molecules did not match, the recipient's prognosis was significantly better than the match.

New leukemia patients and their immune function after chemotherapy were generally considered lower than normal, NK cell immune function such as how the immune reconstitution after chemotherapy, NK cell activity and the prognosis of leukemia. Current the research of acute leukemia immune reconstruction after chemotherapy was less abroad, and T cells' function was recovery fast with the progress of chemotherapy, but another study was opposite. However the quantity of NK cells in two studies after chemotherapy can be gradually increased. But it significantly increased after drug withdrawal. In both studies, none of them related to NK functional activity of human leukemia. Studies [10] have shown that after a course of standard chemotherapy NK cell activity and quantity were higher than before, but by the domestic and foreign literature lack of different stages of chemotherapy NK cell activity. The results of this study showed that the number and activity of NK cells in newly diagnosed leukemia patients were lower than remission children, and the duration of remission, both gradually increased, respectively 6-9 months and 1 year later increased, like the increase in the number of activity to be ahead of the rise, but after two years of remission is still lower than normal, indicating that NK immunization functional recovery was a slower process, similar to the literature [11]. Yoda [10] thought that NK cell activity recovery complex is a slow process, even in patients with leukemia complete remission, but the activity of NK cells can not yet achieve positive constant level. Suggesting that the low NK cell immune function and leukemia itself and the inhibition of chemotherapy, but the latter part of the activity In the future, the relationship between chemotherapy and immunological function of the study may be to answer this question, it may be the length of chemotherapy in the choice of treatment to provide a basis. In our limited study data, we found that neonatal leukemia group, ANLL children with NK cell activity higher than ALL children, but the number of ALL NK cells than ANLL. This may be prompted from the one hand, children with ANLL leukemia Low or absent expression of HLA-1 molecules in cells, NK receptor KIR is not activated, thereby activating the NK Cells, and leukemic cells of ALL patients with high expression of HLA-1 molecules, NK cells in a suppressed state. The amount of small, still need to expand the sample to be confirmed. Some scholars have reported that NK cell activity and tumor prognosis has a great relationship. Zhu [12] by studying NK cells In hepatocellular carcinoma and the relationship between CD56+ cell infiltration density and the survival of patients with hepatocellular carcinoma. Gong [13] confirmed that the prognosis and survival time of NK/T cell infiltration group were significantly better than NK/T cell infiltration group through discussion NK/T cells in squamous cell carcinoma of the lung infiltration and the survival and prognosis of patients with lung squamous cell carcinoma. In hematopoietic stem cells, after transplantation, the body's immune gradually restored, NK cells as a natural immune cells plays an important role. The faster recovery of cytotoxic activity, the less chance of recurrence of transplant patients, NK cell activity and the prognosis of transplantation is very large relationship [14]. However, the relationship between the NK cell function and prognosis of leukemia after chemotherapy is not reported, this study because the research cycle Short, a small number of patients, not enough to determine the relationship between the two, to be studied samples of the expansion and prospective study. But immune function and tumor prognosis may be related, so reduce the intensity of chemotherapy, strengthen immunotherapy is the direction of leukemia treatment research in future.

NK cells clearance of residual leukemia has been the important role of hematopoietic stem cell transplantation and it confirmed by the success. How to mention NK cells function of high acute leukemia patients, there are many studies improved its activation activity and quantity. The timing of treatment should be based on the choice of the

body's immune function, before and after the end of the increase in autoimmune function. It should be just while this is currently the majority of research options, but there is also much early implementation of immunotherapy. NK cell activity detection method is an important means of NK cell research, there are a variety of methods, but each has its advantages and disadvantages. Morphological method is simple, but only suitable for clinical qualitative. The isotope method and lactate dehydrogenase enzyme release method can be quantitative, and therefore it is a routine laboratory method. But the reproducibility is not high, poor stability. Fluorescence method has good specificity and can be determined volume, simple operation, can be used as a substitute for the conventional method of isotope, but the operation is complex. The detection method of cell-active-port 1 has the advantages of rapidity, sensitivity and fewer effector cells required, and it is applied in recent years. The method first reported by foreign countries [15], nearly 2 years of domestic gradually related reports [4], NK cell activity based on CD107a labeled method of detection of hemophagocytic lymphangiosis patients NK. Lysosome-associated membrane protein-1 (LAMP-1 or CD107a) accounts for approximately 50% of the lysosomal membrane protein. It is a type of highly glycosylated protein. When NK cells and K562 cells co-cultured to play a cell activity function, lysosomal-wrapped toxic granule contents released, leading to the death of target cells. With the degranulation of cell active substances, CDl07a molecules are transported by the lysosomal membrane to the cell membrane surface. Thus, CD10a molecules were positively expressed in NK cells May represent NK cells having cytotoxic activity. But not by target cells to stimulate the Nl (cell surface almost does not express CD107a.It was found that the expression of CD107a in NK cells was positively correlated with the NK activity measured by the 51Cr release assay of NK cells, the expression of cytokines is positively correlated and represent the activity of NK. This study showed no stimulation by target cells NK cell surface expression of CD107a molecules rarely, at 0.00% -3.91%, but stimulated by K562 cells can be large expression in the cell membrane surface. To confirm the reliability of NK cell degranulation assay for NK cell viability, comparing with the traditional method of measuring the activity of NK cells by LDH cell activity, we found that the two detection methods have better (correlation coefficient: R=0.754). But compared with the traditional detection method, CD107a labeled NK detection method cell activity, the results of stability easy operation, the effect of fewer cells, the characteristics of high sensitivity.

NK clearance of residual leukemia has been the important role of hematopoietic stem cell transplantation has been confirmed by the success, how to mention high acute leukemia patients' NK cell function. There are more studies improved its activation activity and quantity. Treatment should be based on the choice of the body's immune function, before and after ending of the chemotherapy improving their immune function should be appropriate. This is currently the choice of most studies, but there is also much early implementation of immunotherapy. And its advantages to be explore deeper. In this study, because of the short study period, the sample size is small, the observation time is short. In the future we will expand the sample study and conduct prospective studies.

5. Conclusion

In conclusion, we proved that the the detection of NK cell activity with CD107a-based flow cytometry is a sensitive, reliable and easy way, and it was positively correlated to LDH release method. We also found that the quantity and activity of NK cells in newly diagnosed leukemia patients were lower than those who in the normal control group, and gradually increased with the prolongation of time, such as 6 months and one year after complete remission, but even two years later, it is still lower than normal control group.

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