THE RELATIONSHIP BETWEEN THE ABSOLUTE VALUE OF PERIPHERAL BLOOD LYMPHOCYTES IN PATIENTS WITH ACUTE MYELOID LEUKEMIA +21 DAYS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION AND RECURRENCE AND OTHER CLINICAL EFFICACY

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ABSTRACT

Objective*: To analyze the relationship between the absolute value of peripheral blood lymphocytes (ALC21) and recurrence and other clinical efficacy +21 days after allogeneic hematopoietic stem cell transplantation (allo-HSCT) in patients with acute myeloid leukemia (AML).*

Methods: *AML patients undergoing allo-HSCT admitted to the Department of Hematology in our hospital from January 2015 to January 2020 were retrospectively reviewed. 4 ml of fasting venous blood was collected from all patients 21 days after transplantation. The absolute value of peripheral blood lymphocytes (ALC21) of all patients was detected using flow cytometry. According to their ALC21 levels, they were divided into an ALC21 high-expression group (ALC21≥0.50×10^9/L, n=63) and an ALC21 low-expression group (ALC21<0.50×10^9/L, n=43). All patients were followed up for five years, and indicators including the recurrence rate, overall survival rate (OS), Epstein–Barr virus (EBV) infection, cytomegalovirus (CMV) infection, acute graft-versus-host disease (aGVHD), and disease-free survival rate (DFS) were counted. The correlation between ALC21 and two-year OS and DFS was analyzed using Pearson linear correlation, and the relationship between ALC21 level and recurrence and other clinical efficacy in AML patients after allo-HSCT was analyzed.*

Results: *The total infection rate of patients in the ALV21 high-expression group was 33.33%, which was significantly lower than the 62.79% in the ALC21 low-expression group, and the difference was statistically significant (P<0.05). There was no significant difference in ERV and CMV infection between the two groups (P>0.05). Out of a total of 106 patients in this experiment, 25 patients developed acute graft-versus-host disease (aGVHD) with a total incidence of 23.58%; there were 10 cases of grade I-II aGVHD with an incidence rate of 9.43%; and there were 14 cases of grade III-IV aGVHD with an incidence rate of 13.21%. The rate of aGVHD in the ALC21 high-expression group was 17.46%, which was significantly lower than the rate of 32.56% in the ALC21 low-expression group. The difference was statistically significant (P<0.05). This experiment was followed up for up to five years after transplantation and found that the post-transplant survival rate was 61.32%, and the post-transplant recurrence rate was 23.10%. Among them, 45 patients (71.43%) in the ALC21 high-expression group survived for five yearshigh-expression, and the post-transplant recurrence rate was 13.33%. Twenty patients (46.51%) in the ALC21 low-expression group survived for five years, and the recurrence rate after transplantation was 45.00%. The five-year survival rate, two-year OS, and DFS in the ALC21 high-expression group were significantly higher than those in the ALC21 low-expression group, and the five-year recurrence rate in the ALC21 high-expression group was significantly lower than that of the ALC21 low-expression group, and the difference was statistically significant (P<0.05). ALC21 was significantly positively correlated with two-year OS and DFS (r=0.526, 0.612, and P values were both <0.01 or <0.05).*

Conclusion: *The ALC21 level after allo-HSCT in AML patients is closely related to the patient's infection, aGVHD occurrence, two-year OS, and DFS, which can predict the disease recurrence and prognosis after transplantation early, and can be widely used in clinical practice.*

Keywords: *Acute myeloid leukemia; allogeneic hematopoietic stem cell transplantation; peripheral blood lymphocytes; recurrence; clinical efficacy.*

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Introduction

Acute myelogenous leukemia (AML) is a clonal malignant proliferative disease of myeloid blasts of the hematopoietic system. The autoimmune function of leukemia patients declines, and there is the phenomenon of immune escape of leukemia tumor cells in patients, including all non-lymphocyte sources; it is the most common acute leukemia in adults[1, 2]. Presently, AML is mainly treated with allogeneic hemopoietic stem cell transplantation (allo-HSCT): it is one of the main treatment methods, but about 70% of patients who have achieved remission eventually relapse

and evolve into refractory leukemia, thus leading to treatment failure and death. Disease recurrence is one of the leading causes of transplant failure and patient death[3]. Therefore, early prediction of recurrence after metastasis and targeted treatment can significantly reduce the recurrence rate of the disease, significantly improve the prognosis after transplantation, and prolong the life cycle of patients. Therefore, finding a highly sensitive and specific biological standard for early assessment of disease recurrence after allo-HSCT is of great significance for early diagnosis of recurrence and prolonging the life cycle of patients[4].

The absolute value of peripheral blood lymphocyte +21 days after transplantation (ALC21) represents the recovery rate of lymphocytes after transplantation, which can indirectly reflect the immune reconstitution of the body after transplantation, indicating the graft-versus-leukemia effect (GVL) is strong or weak, but the theory that there is a predictive effect on the recurrence of the primary disease is controversial[5-7]. This experiment retrospectively analyzed the AML patients who underwent allo-HSCT in the Department of Hematology of our hospital from January 2015 to January 2016, aiming to analyze the relationship between ALC21 and recurrence and other clinical efficacy after allo-HSCT in AML patients.

Materials and methods

General information

Patients with acute myeloid leukemia who underwent allo-HSCT admitted to the Department of Hematology in our hospital from January 2015 to January 2020 were retrospectively reviewed.

Inclusion criteria:

• All patients met the diagnostic criteria for AML in the "Acute Myeloid Leukemia Classification" revised by WHO in 2016[8];

• All patients were diagnosed as having AML by morphological, immunological, molecular biology, and cytogenetic examinations;

• The clinical data of all patients were complete without loss;

• All patients underwent allo-HSCT in the Department of Hematology in our hospital;

• Patients and their families were informed and signed informed consent forms.

Exclusion criteria:

• The patient had serious disorders of important organs including the heart, liver, or kidney;

• The patient had a malignant tumor;

• The patient had autoimmune disease;

• The donor had infectious diseases such as hepatitis B surface antigen positive or hepatitis C antibody positive, syphilis, or HIV infection;

• Patients or family members refused allo-HSCT or this experiment, or terminated this experiment for other reasons.

Observation indicators

Serum test

Four ml of fasting venous blood was collected from all patients 21 days after transplantation, left at room temperature for 20 minutes, and centrifuged at 3000 r/min for 10 minutes. The serum was carefully separated and put in a -70 °C environment for later use to avoid repeated freezing and thawing. The absolute value of peripheral blood lymphocytes (ALC21) in all patients was detected using flow cytometry.

ALC21≥0.50×10^9/L was the high-expression group of ALC21, and ALC21<0.50×10^9/L was the low-expression group of ALC21. All patients were followed up for five years, and indicators including the recurrence rate (primitive or immature cells in peripheral blood and/or extramedullary infiltration of leukemia cells after transplantation), over-all survival (OS), Epstein–Barr virus (EBV) infection, cytomegalovirus (CMV) infection, acute graft-versus-host disease (aGVHD), and disease-free survival (DFS) were counted.

Statistical methods

The data in this study were analyzed using the SPSS20.0 software package. All measurement data were compared by ($\bar{x}\pm s$), and the comparison between groups was by t test; the enumeration data were all expressed as percentages, and the comparison between groups was by χ^2 test.

The correlation between ALC21 and two-year OS and DFS was analyzed using Pearson linear correlation, and the statistical results were statistically significant at $P<0.05$.

Results

General data analysis of patients in the two groups

There were 106 patients in this experiment, consisting of 54 males and 52 females. There were 63 cases in the ALC high-expression group, including 32 males and 31 females. Their average age was

(35.06±9.78) years, and their average BMI value was (20.11±0.98) Kg/m^2. There were 43 cases in the ALC low-expression group, including 22 males and 21 females. Their average age was (35.11±8.98) years, and their average BMI value was (20.05±0.88) Kg/m^2. There was no significant difference in age, gender, BMI value, donor and recipient gender, disease risk, minimal residual disease (MRD) before metastasis, or ABO blood type between the two groups (P>0.05). See Table 1 and 2.

Comparison of the occurrence of GVHD between the two groups

Out of a total of 106 patients in this experiment, 25 patients developed aGVHD with a total incidence rate of 23.58%; there were 10 cases of grade I-II aGVHD with an incidence rate of 9.43%; and there were 14 cases of grade III-IV aGVHD with an incidence rate of 13.21%.

The incidence of aGVHD in the ALC21 high-expression group was 17.46%, which was significantly lower than the 32.56% in the ALC21 low-expression group, and the difference was statistically significant (P<0.05). See Table 4.

Group	Case	Disease risk degree (case)			Sex (case)		BMI value (Kg/m^2)	Donor and recipient gender (case)			
		LR	IR	HR	Male	Female		MFM	MFF	FFM	FFF
ALC21 high-expression group	63	9	26	28	32	31	20.11±0.98	11	16	17	18
ALC21 low-expression group	43	3	15	25	22	21	20.05±0.88	18	11	10	5
t/χ^2		2.434			0.001		0.322	7.01			
P		0.296			0.970		0.747	0.071			

Table 1: General data analysis of patients in the two groups ($\bar{x}$±s).
LR: low-risk; IR: intermediate-risk; HR: high-risk; MFM: male for male; MFF: male for female; FFM: female for male; FFF: female for female.

Group	Case	MRD	Age (year)	ABO Blood type (case)				CR (case)		
				C	ABO-major compatible	ABO-minor compatible	ANC	CR1	CR2	CR3
ALC21 high-expression group	63	1.26±0.36	35.06±9.78	36	12	9	6	39	18	6
ALC21 low-expression group	43	1.32±0.41	35.11±8.98	20	11	9	3	29	11	3
t/χ^2		0.796	0.026	1.909				0.4001		
P		0.427	0.978	0.591				0.818		

Table 2: General data analysis of patients in the two groups ($\bar{x}$±s).
C: compatible; ANC: all non-compatible.

Comparison of infection between the two groups

The total infection rate of patients in the ALV21 high-expression group was 33.33%, which was significantly lower than the 62.79% in the ALC21 low-expression group, and the difference was statistically significant (P<0.05). There was no significant difference in ERV and CMV infection between the two groups (P>0.05). See Table 3.

Group	Case	EBV infection	CMV infection	Total infection
ALC21 high-expression group	63	12	9	21 (33.33%)
ALC21 low-expression group	43	14	12	27 (62.79%)
χ^2		2.520	2.985	4.445
P		0.114	0.084	0.035

Table 3: Comparison of infection between the two groups (case, %).

Group	Case	I-II grade aGVHD	III-IV grade aGVHD	aGVHD total occurrence
ALC21 high-expression group	63	4	6	11
ALC21 low-expression group	43	6	8	14
χ^2		1.729	1.838	3.232
P		0.188	0.175	0.072

Table 4: Comparison of the occurrence of GVHD between the two groups (case, %).

Analysis of the survival of patients in the two groups after transplantation

The experiment was followed up for up to five years after transplantation and found that the post-transplant survival rate was 61.32%, and the

post-transplant recurrence rate was 23.10%. Among the patients, 45 (71.43%) survived for five years in the ALC21 high-expression group, and the post-transplant recurrence rate was 13.33%.

Twenty patients (46.51%) in the ALC21 low-expression group survived for five years, and the recurrence rate after transplantation was 45.00%. The five-year survival rate, two-year OS, and DFS in the ALC21 high-expression group were significantly higher than those in the ALC21 low-expression group, and the five-year recurrence rate in the ALC21 high-expression group was significantly lower than that of the ALC21 low-expression group, and the difference was statistically significant (P<0.05). ALC21 was significantly positively correlated with two-year OS and DFS (r were 0.526, 0.612, and P values were both <0.01 or <0.05). See Table 5 and 6.

Group	Case	Five-year survival rate	Five-year recurrence	Two-year OS	Two-year DFS
ALC21 high-expression group	63	45	6	74.56±5.72	71.26±5.26
ALC21 low-expression group	43	20	9	51.62±7.84	51.38±7.82
χ^2		6.690	9.410	17.418	15.659
P		0.010	0.002	<0.001	<0.001

Table 5: Comparison of survival after transplantation between the two groups (cases, %).

Indexes		Two-year OS	Two-year DFS
ALC21	*r*	0.526	0.612
	P	0.001	0.023

Table 6: Correlation analysis between ALC21 and two-year OS and DFS.

Discussion

AML is a highly specific hematological malignant disease, with the main feature of myeloid leukemia cells derived from malignant hematopoietic stem and progenitor cells, and it is largely accumulated in the bone marrow. It is the most common acute leukemia in adults and seriously endangers their lives[9]. The allo-HSCT treatment regimen is an effective treatment for AML. Studies have confirmed that allo-HSCT is safe and effective in the treatment of leukemia patients[10].

Allo-HSCT can kill leukemia cells through myeloablative pretreatment and can also clean up MRD through the graft anti-leukemia (GVL) effect[11]. Enhancing the intensity of pretreatment can increase the toxicity of pretreatment and treatment-related mortality to varying degrees. Therefore, enhancing the GVL effect through immunotherapy has become a reasonable choice to reduce the recurrence rate[12]. AML patients can have lymphocyte subpopulations, imbalances in the proportion of lymphocytes, and immune mechanism disorders, which are closely related to the pathogenesis of leukemia and can also play a certain role in the outcome of leukemia patients[13]. Therefore, the changes of lymphocytes in the body of AML patients indirectly reflect the body's anti-tumor immune function, and the immune status of AML patients can be assessed and detected by detecting the proportions and changes of lymphocytes in each subgroup. EBV and CMV are the more common infection types after AML surgery. EBV mainly invades human B lymphocytes, while CMV can involve various organs such as the lung and central nervous system, both of which seriously threaten the efficacy and survival of patients.

The body's immune function is reduced due to patients requiring high-dose chemotherapy and immunosuppressant-related treatments after allo-HSCT, and the virus cannot be effectively eliminated, which can easily lead to EBV and CMV infection and seriously affect the subsequent treatment and prognosis of patients[14].

In this experiment, the total infection rate of patients in the ALV21 high-expression group was 33.33%, which was significantly lower than the 62.79% in the ALC21 low-expression group, and the difference was statistically significant (P<0.05). There was no significant difference in ERV and CMV infection between the two groups (P>0.05). Out of a total of 106 patients in this experiment, 25 patients developed aGVHD with a total incidence rate of 23.58%; there were 10 cases of grade I-II aGVHD with an incidence rate of 9.43%; there were 14 cases of grade III-IV aGVHD with an incidence rate of 13.21%. The incidence of aGVHD in the ALC21 high-expression group was 17.46%, which was significantly lower than the 32.56% in the ALC21 low-expression group, and the difference was statistically significant (P<0.05). This shows that the lymphatic count after transplantation has a significant impact on the prognosis, while preserving the effect of GVL and controlling GVHD, thereby increasing the success rate of transplantation, similar to the results of the study by Gao et al[15].

Postoperative recurrence is the main cause of death in AML patients, which seriously endangers the life and health of patients. Follow-up in this experiment up to five years after transplantation

found that the survival rate after transplantation was 61.32%, and the recurrence rate after transplantation was 23.10%. Among them, 45 cases in the ALC21 high-expression group survived for five years, and the recurrence rate after transplantation was 13.33%. Twenty cases (46.51%) in the ALC21 low-expression group survived for five years, and the recurrence rate after transplantation was 45.00%. The five-year survival rate, two-year OS, and DFS of the ALC21 high-expression group were significantly higher than those of the ALC21 low-expression group. The five-year recurrence rate of the ALC21 high-expression group was significantly lower than that of the ALC21 low-expression group, and the difference was statistically significant ($P<0.05$). ALC21 was significantly positively correlated with two-year OS and DFS (r was 0.526 and 0.612, respectively, P values were both <0.01 or <0.05), showing that the ALC21 level after allo-HSCT in AML patients is closely related to the two-year OS and DFS, which indirectly indicates that the rapid recovery of early lymphocytes after transplantation can significantly improve the long-term survival of patients after transplantation.

In conclusion, the ALC21 level after allo-HSCT in AML patients is closely related to the patient's infection, aGVHD occurrence, two-year OS, and DFS, which can predict disease recurrence and prognosis after transplantation early and can be widely used in clinical practice.

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