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Correlation study on CD7, CD34, CD56 and HLA-DR expressions and its prognosis among patients with acute myeloid leukemia.

Shishan Xiao, Hongqian Zhu*

Hematology Department of Guizhou Provincial People's Hospital, Guiyang city Guizhou Province, PR China

Abstract

Objective: To explore correlations between CD7, CD34, CD56 and HLA-DR expressions and its prognosis among patients with acute myeloid leukemia.

Methods: 225 patients with Acute Myeloid Leukemia (AML) with initial treatment and complete data in our hospital from Jan 2013 to Dec 2016 were selected as study objects. 30 healthy volunteers who took part in the marrow test but were not detected for leukemia in the same period were selected as control patients. Fresh bone marrow of all patients was abstracted, from which single cell was separated. Flow cytometer detection was given after using monoclonal antibody of living cells immunofluorescent. CD7, CD34, CD56 and HLA-DR expressions of bone marrow among patients were detected. Fluorescent antibody staining positive cells were equal or greater than 20%, they were positive; equal or greater than 50%, they were high expression. Various immunophenotypes distribution in AML patients with different types was given statistics. Complete Remission (CR) rate of different immunophenotype positive and negative patients were given statistics. Mean survival of different immunophenotype positive and negative patients were given statistics.

Results: There was no significant difference of leukemia patient and healthy volunteer in the aspects of age, sex, past medical history with relevant comparability (p>0.05). Antigen expression rate, from the high level to low were HLA-DR (58.22%), CD349 (50.67%), CD56 (30.67%), CD (723.11%). CR rate of patients in CD7, CD34, CD56 and HLA-DR positive groups were lower than negative group significantly, there were statistical differences between two groups (P<0.05). MST among patients in CD7, CD34, CD56 and HLA-DR positive group were lower than negative group significantly, there were statistical differences between two groups (P<0.01).

Conclusion: Complete remission rate of CD7, CD34, CD56 and HLA-DR antigen expression positive among patients with Acute Myeloid Leukemia (AML) is low, mean survival is short and prognosis is poor.

Keywords: Acute myeloid leukemia (AML), Immunophenotype, Prognosis.

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Introduction

Leukemia is a kind of malignant clone of hemopoietic stem cell disease, the reasons are apoptosis of leukemia cells are restricted, self-renewal of cells are higher than common cells and cell proliferation cannot be controlled. Differentiation processes of cells have disorders, which stop the growth of cells. An amount of leukemia cells spread and infiltrate into bone marrow and other hematopoietic tissue. Hematopoiesis of normal bone marrow is inhibited greatly [1,2]. AML as the most common type of leukemia has characteristics of high incidence rate, short history, which cause heavy economic burden for society [3]. In China, incidence rate of leukemia reaches to 2.76/10 ten thousands, whereas in European and American area, reaches to 3.4/10 ten thousands [4]. However, the acute myelogenous leukemia is mainly a recognizable acute leukemia subtype, secondary to chemotherapy, radiotherapy or acute leukemia with definite environmental or occupational exposure history. Main clinical manifestations are anemia, bleed, spleen lymphadenectasis, and infiltration of central nervous system and so on. In recent years, with environmental pollution and population aging becoming more severe, incidence of leukemia increases gradually. Our nation mainly uses the classification standard of France, America and Britain Cooperative Group (France, America, Britain, and FAB) in the 1970's for current initial analysis of leukemic. WTO classifies patients according to the clinical feature, morphology, cytochemistry, immunology, molecular biology, cell genetics and so on, namely MICM classification. Immunophenotypes of leukemia can identify cells which cannot be identified by morphology, and it can promote diagnosis for leukemia and play a certain prognosis judgment [5,6]. There are reports showing that CD7, CD34, CD56 and HLA-DR expressions have a certain correlations with prognosis of patients with leukemia [7-9]. Therefore, this study selects 225 patients with

Acute Myeloid Leukemia (AML) in our hospital from Jan 2013 to Dec 2016 as study objects to explore CD7, CD34, CD56 and HLA-DR expression antigen and its relations with clinical effects and prognosis. It is reported as follows.

Data and Methods

General data

This study selected 225 patients with Acute Myeloid Leukemia (AML) with initial treatment and complete data in our hospital from Jan 2013 to Dec 2016 as study objects. 30 healthy volunteers who took part in the marrow test but were not detected for leukemia in the same period were selected as control patients; there were 134 males and 91 females. The age was from 15-82 y old. The average age was 41.58 ± 7.18 y old. According to the type of morphology, immunology, cytogenetics and molecular biology of cells, namely MICM type, the types of AML are 54 cases with M1, 109 cases with M2, 30 cases with M3, 14 cases with M4, 6 cases with M6 and 4 cases with M7. In the healthy volunteers, there are 14 male cases, 16 female cases, of which the age is from 20-80 y and average year is 39.24 ± 8.42 y. There is no significant difference of leukemia patients and healthy volunteers in age, sex and other clinical data (P>0.05). Under consent of Ethics committee in our hospital, all patients signed informed consent and passed ethic justification.

Treatment methods

Induction program of initial treatment: This study has selected all-trans retinoic acid or arsenious acid induction treatment for patients with M3, TA induction scheme: 20 mg/m^2/d pirarubicin in intravenous drip; 100~150 mg/m^2/d cytarabine in intramuscular injection through over two treatment courses; DA induction scheme: 45 mg/m^2/d daunorubicin in intravenous drip, cytarabine with above same dose; HA induction scheme: 4~6mg/m^2/d harringtonine in intravenous drip, cytarabine with above same dose; MA induction scheme: 6mg/m^2/d mitoxantrone in mainline, cytarabine with above same dose for other Acute Myelogenous Leukemia (AML) patients. Patients with condition changes and intolerance were excluded. Bone marrow and blood picture were detected and curative effects were identified after two treatment courses.

Standards of efficacy determination

Taking hemopathy diagnosis and therapeutic effects standards as references, treatment effects were given determination [10]. The Complete Remission (CR) referred to disappear of clinical symptoms and signs. In blood routine examination detection, the level of neutrophil was equal to or greater than 1.5 × 109/L. The level of platelet was equal or greater than 100 × 109/L. There were no abnormal leukemia cells in blood smear. In morphology of bone marrow, the level of myeloblast and promyelocyte were equal to or less than 5%. Morphology of bone marrow cells among patients with M3, except the level of myeloblast and promyelocyte were equal to or less than 5%, there were no Auer corpuscle in bone marrow level and morphology of erythrocyte and megakaryocyte. There was no infiltration of leukemia out of bone marrow.

Detection of immunophenotype

This detection selected 3 ml fresh bone marrow samples of all patients, used heparin anti-freezing to separate single cell, adopted living cell monoclonal antibody immunofluoresent label to give flow cytometer detection (Epics XL type, Coulter, USA) for detecting level of several immunophenotype in bone marrow of patients. CD7, CD34, CD56 and HLA-DR single antibody were products of Immunotech Company in France. Criterion of detection results showed that fluorescent positive cells were equal to or greater than 20%, they were positive; equal to or greater than 50%, they were high expression.

Main prognosis indexes

Main prognosis indexes included, first, reaching to number of CR patients (CR rate); second, Median Survival Time (MST) of patients was recorded, namely the corresponding survival time when cumulative survival rate was 0.5, which showed only 50% individual can live surpass this time.

Observation indexes

First, general data of sex, age, FAB type of all patients were given statistical analysis; second, detection results of immunophenotype of patients were recorded; third, the relations between immunophenotype and AML phenotype were recorded; fourth, correlations between results of immunophenotype and prognosis of patients.

Data statistics

This study used SPSS 17.0 software to deal with all data. Measurement data used $\bar{x} \pm s$ form and t-test; Enumeration data used n, %form and χ^2 test. P<0.05, there were statistical differences.

Results

General comparison of clinical data between AML patients and healthy volunteers

Relations between various immunophenotype among AML patients types: As shown in Table 1, AML patients and healthy volunteers have no significant differences in age, sex, diabetes, hypertension, high blood fat and other past medical history with relevant comparability (P>0.05).

Table 1. *Comparison of general clinical data between AML patients and healthy volunteers.*

	Leucocythemia group	Healthy volunteer group	χ^2 or T value	P value
Sex (M/F)	134/91	43028	0.56	0.454
Age	41.58 ± 7.18	39.24 ± 8.42	1.642	0.102
Diabetes	27	3	1	0.102
Hypertension	30	4	0	1
High blood fat	32	4	0.017	0.896

Relations between immune phenotypes and subtype of AML patients

It was found in the marrow test of 30 healthy volunteers that CD34, CD56, CD7 and HLA-DR are expressed as negative. So, the disturbance of marrow testing of the healthy volunteers could be excluded. Immunophenotype conditions of 225 AML patients were shown in Table 2. The order of antigen expression rate were: HLA-DR (58.22%), CD34 (50.67%), CD56 (30.67%), CD7 (23.11%), of which, HLA-DR had low expression among AML patients with M3, type expressions of other patients were over 50%; CD56 had no expression among patients with M7 *in vivo*, low expressions among other type patients *in vivo*. CD7 had no expression among patients with M7 *in vivo*, low expressions among other type patients *in vivo*. The χ^2 value and P value of CD34, CD56 and HLA-DR *in vivo* expressions to CD7 expressions were 36.692, 0.000; 3.267, 0.071; 57.478, 0.000, respectively; results indicated that there was significant difference between the CD7 expressions and CD34 and HLA-DR expressions of the leucocythemia patients ($P<0.05$); the χ^2 value and P value of CD56 and HLA-DR *in vivo* expressions to CD34 expressions were 18.65, 0.000; 2.589, 0.108, respectively; the results indicated that there was significant difference between CD34 and CD56 expressions of the leucocythemia patients ($P<0.05$); the χ^2 value and P value of CD56 and HLA-DR expressions were 34.596, 0.000. Respectively, which indicated that there was significant difference between CD56 and HLA-DR expressions of the leucocythemia patients ($P<0.05$).

Relations between antigen type of 225 AML patients and clinical effects

154 patients in 225 AML patients were given chemotherapy and therapeutic effects according to standard chemotherapy regimen. They were divided into the positive group and the negative group according to various antigen expression conditions. Compared with CR rate after chemotherapy as Table 3, the CR rates of CD7, CD34, CD56 and HLA-DR among patients in positive group were lower than the negative group, there were statistical differences between two groups ($P<0.05$).

Relations between AML patient's antigen type and OS of patients

AML patients in 154 cases were given chemotherapy according to standard chemotherapy regimen. They were divided into the positive group and the negative group according to various antigen expression conditions. Compared with MST rate after chemotherapy as Table 4, the MST rates of CD7, CD34, CD56 and HLA-DR among patients in positive group were lower than the negative group, there were statistical differences between two groups ($P<0.001$).

Table 2. *Relations between various immunophenotype and subtype of AML patients (case (%)).*

CD	Constituent ratio of types							Positive expression (case)	Percentage (%)
	M1 (n=54)	M2 (n=109)	M3 (n=30)	M4 (n=14)	M5 (n=8)	M6 (n=6)	M7 (n=4)		
CD7	4 (25.93)	26 (23.85)	17 (56.67)	2 (14.29)	3 (37.50)	0 (0.00)	0 (0.00)	52	23.11
CD34	16 (29.63)	71 (65.14)	5 (16.67)	9 (64.29)	6 (75.00)	4 (66.67)	3 (75.00)	114	50.67
CD56	21 (3.89)	42 (38.53)	1 (3.33)	2 (14.29)	1 (12.50)	2 (33.33)	0 (0.00)	69	30.67
HLA-DR	25 (46.30)	76 (69.72)	4 (13.33)	12 (85.71)	7 (87.50)	4 (66.67)	3 (75.00)	131	58.22

Table 3. *Relations between antigen type of 225 AML patients and clinical effects (cases (%)).*

CD	Positive number	Positive CR rate (%)	Negative rate	Negative CR rate (%)	χ^2 value	P value
CD7	37	15 (40.54)	117	74 (63.25)	5.942	0.015
CD34	77	38 (49.35)	77	65 (84.42)	21.372	0

CD56	48	26 (54.17)	106	78 (73.58)	5.682	0.017
HLA-DR	91	47 (51.65)	63	49 (77.78)	10.826	0.001

Table 4. *Relations between AML patient's antigen type and OS of patients ($\bar{x} \pm s$, month).*

CD	Positive number	MST (month)	Negative number	MST (month)	χ^2 value	P value
CD7	37	19.86 ± 7.34	117	40.58 ± 9.18	12.513	0
CD34	77	20.94 ± 8.92	77	49.69 ± 10.58	18.23	0
CD56	48	21.09 ± 8.27	106	41.97 ± 9.57	13.063	0
HLA-DR	91	18.36 ± 6.79	63	38.36 ± 7.79	16.914	0

Discussion

There are great differences of clinical manifestations, immune types, morphology of leukemia cells and its sensitivity in chemotherapy among leukemia patients. Elaboration, reification and individualization of conditions among leukemia patients are very important; this process needs different detection methods [11-13]. At present, the most common detection of chemotherapy is morphology, immunology, molecular cytogenetics and biological typing. But this routine examination is difficult for identifying atypical and undifferentiated of cellular morphology in leukemia. Flow cytometry as one of important technology for analyzing bone marrow and blood cells, most of them use fluorescence labelling monoclonal antibody to mark the corresponding antigen of various cells and are given multi-parameter qualitative and quantitative analysis [14,15]. The results of flow cytometer are used to analyze specific antigen of cytomembrane and cytoplasm among leukemia patients, which can observe specific information of detecting cellular development and differentiation. It has simple, rapid, sensitive, reliable, easily repeated features, which are used for detecting malignant tumor of blood system [16]. Leukemia patients are given immune typing detection, which can judge specific antigen expression of patients effectively to a great extent, provide scientific basis for individual diagnosis of leukemia, treatment program and prognosis etc.

CD34 antigen is symbolic antigen of HSC and CFU-E. Expression in single cell nucleus in normal bone marrow is below 3%, which is an available flag of primary cell detection. There are documents report that prognosis of AML patients with CD 34 positive are poor [17]. CD7 antigen is a kind of single strand glycoprotein, its expression in thymocyte in early time and T cells has certain contagious distribution. There are reports show that leukemia patients with CD7 positive have poor prognosis and their overall survival is short [18]. But there are CD7 expression has no direct relations with prognosis of patients [19]. Expression rate of CD56 in NBLC is from 10%-15%. CD56 positive AML patients have low CR rate relatively, accompanying with poor prognosis usually [20]. HLA-DR is main tissue compatibility antigen molecule of human HLA, which has expressions in B lymphocytes and monocytes, and antigen for identifying lymphoma and leukemia [21]. 225 AML patients admitted in our hospital, the order of antigen expression rate from high to low are: HLA-DR (58.22%), CD34 (50.67%), CD56 (30.67%), CD7 (23.11%), of which, HLA-DR has low expression among AML patients with M3 type, high expressions in other phenotypes. CD34 phenotype has low expressions among AML patients with M1 and M2 type, expressions in other patients type are over 50.00%. CD56 phenotype has no expression among patients with M7 *in vivo*, low expressions in other type patients *in vivo*. CD7 has no expression among patients with M7 *in vivo*, low expressions relatively in other type patients *in vivo*, which show that immunophenotypes have different expressions among different patients. It may become pathway of individual diagnosis. CR rate, MST of CD7, CD34, CD56 and HLA-DR of patients in positive group are lower than negative group, there are statistical differences between two groups ($P<0.05$). The results are consistent with paper conclusions of Ling that poor prognosis and CR rate of CD34 positive patients, with paper of Wenyi that low CR rate of CD7, CD34 positive patients in AML [22,23]. Of which, poor prognosis of positive patients may be related to strong invasion of AML cells in CD34 positive, which can influence effects of AML chemotherapy. CD7 and CD 56 positive AML patients are not sensitive to chemotherapy results, which often occurs in early stage of Leukemia. There are studies about HLA-DR which show that recurrence rate is still high after HLA-DR positive patients reach to CR. Some HLA-DR patients, of which, about 4% patients recur after complete remission [24]. HLA-DR expressions of patients are given detection again. HLA-DR expression transforms into positive, which show HLA-DR positive means poor prognosis. It is similar to this study.

At present, the study on the relation between the expressions of different antigens and the prognosis of the leucocythemia patient only rests on surface phenomena. In this study, the marrow antigen expressions of 225 leucocythemia patients and 30 healthy volunteers were compared and the relations between positive expressions of different antigens and the clinical effect and prognosis of the patients were further observed. The sample size was relatively great and antigen types were relatively broad, including relatively associated antigens among different previous studies. The purpose was to provide certain assistance for personalized treatment of leucocythemia.

In conclusion, complete remission of CD7, CD34, CD56 and HLA-DR antigen positive expression among AML patients are low. Mean survival is short. Prognosis is poor. Because there are a small number of samples and the treatment methods for different patients may be different certainly, which may have certain effect on treatment results, this conclusion still needs more samples, polycentric and larger scale study to confirm relations between immune typing and prognosis of AML patients.

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*Correspondence to

Hongqian Zhu

Hematology Department of Guizhou Provincial People's Hospital

Guizhou Province

PR China