

Original Article

Expression level and clinical significance of PD-1, MMP-2 and SE-Cad in patients with acute myeloid leukemia

Ying Wang, Tao Jia, Yuanxin Zhu, Lei Miao, Jianping Mao, Zhimei Cai

Department of Hematology, The First People's Hospital of Lianyungang, Lianyungang, Jiangsu Province, China

Received November 4, 2019; Accepted December 13, 2019; Epub February 15, 2020; Published February 28, 2020

Abstract: Objective: We aimed to research the expression level and the significance of related clinical application of programmed death-1 (PD-1), matrix metalloproteinase 2 (MMP-2), soluble Ecadherin (SE-Cad) in acute myeloid leukemia (AML). Methods: This study is a prospective cohort study. In total, 88 AML patients were selected as our study subjects (the primary diagnosis group), and 88 healthy people were selected as the subjects in the control group. After receiving the standard chemotherapy, AML patients were further separated into the remission group (37 cases) and the non-remission group (51 cases). The ratio of CD4⁺ T cells, CD8⁺ T cells, CD4⁺/CD8⁺ and the expression level of PD-1 on the surface of CD4⁺ and CD8⁺ T cells were compared. The expression of MMP-2 gene in bone marrow mononuclear cells and the expression rate and quantity of SE-Cad in serum were also analyzed. Pearson correlation method was used to analyze the correlation between the levels of PD-1, MMP-2, SE-Cad and the proportion of bone marrow immature cells. The clinical value of each index in evaluating the prognosis of AML patients was evaluated through ROC curve analysis. The results of K-M survival analysis curve showed the short-term survival rate of different indicators in the primary diagnosis group. Results: The ratio of CD4⁺ and CD4⁺/CD8⁺ in the primary diagnosis group decreased significantly ($P < 0.05$), but the positive rates and content of PD-1 on the surface of CD4⁺ cells, PD-1, MMP-2 and SE-Cad on the surface of CD8⁺ cells in the primary diagnosis group all increased significantly compared with the control group ($P < 0.05$). Besides, the positive rate and level of each index were significantly improved ($P < 0.05$) in the remission group compared with the primary diagnosis group, and no statistical difference was shown between the remission group and the control group ($P > 0.05$). At the same time, the positive expression rate and level of CD4⁺, CD8⁺, CD4⁺/CD8⁺, and PD-1 production on CD4⁺ and CD8⁺ cell surface of the non-remission group were also improved ($P < 0.05$), but statistical differences still exist between the non-remission group and the control group ($P < 0.05$). The results of Pearson correlation showed that the levels of PD-1 on the surface of CD4⁺ cells and CD8⁺ cells, SE-Cad level and MMP-2 level were all positively correlated with the proportion of bone marrow immature cells ($r = 0.875, 0.504, 0.683, 0.680$, respectively). After 2 years of follow-up, 37 cases survived and 51 died, with a survival rate of 42.05%. ROC curve showed that the levels of PD-1 on CD4⁺ and CD8⁺ cells surfaces, SE-Cad level and MMP-2 level in the primary diagnosis group had high predictive value in evaluating the prognosis of AML patients (AUC = 0.834, 0.780, 0.786, 0.784, respectively). The K-M survival analysis showed that the short-term survival time of patients who had negative expression of PD-1, MMP-2 and SE-Cad was significantly longer than that of patients with positive index ($P < 0.05$). Conclusion: PD-1, MMP-2 and SE-Cad may be involved in the occurrence of AML, and they all have certain clinical value in the treatment and prognosis evaluation of AML.

Keywords: Acute myeloid leukemia, programmed death-1, matrix metalloproteinase 2, soluble ecadherin

Introduction

Acute myeloid leukemia (AML) is a kind of acute leukemia, which has the characteristics of rapid onset, swift development and poor prognosis. In recent years, with the development of targeted drugs and molecular markers, the diagnosis, efficacy evaluation and prognosis

evaluation of AML patients have made great progress, but the recurrence rate of AML is still at a high level. The data shows that the 5-year survival rate of AML patients is only 21.4%, and that of patients over 75 years old was only 1% [1-3]. Therefore, to explore new molecular markers and therapeutic targets of AML is of great importance for the diagnosis, curative effect

and prognosis of AML. It was found that the mechanism for tumor immune escape is a recent research priority topic of tumor immunology, and it has been considered as one of the key factors effecting tumor immunotherapy. At present, it is believed that tumor immune escape mechanisms include tumor microenvironment and the tumor cells' modification of themselves [4]. PD-1 together with its ligand PD-L1 are negative immunoregulatory factors discovered in recent years, and the activation of PD-1/PD-L1 can prevent the activation of T cells in tumor microenvironment, thus promoting tumor cells to escape surveillance and killing mechanism induced by the immune system, and weakening the anti-tumor function of the body [5]. Research shows that PD-1 is highly expressed in many solid tumors including breast cancer and prostate cancer [6], and the research about PD-1/PD-L1 immunosuppressant application in blood tumors has already been tested in the clinical trial stage; so exploring the expression of PD-1 in AML and its clinical value in evaluating prognosis can provide theoretical basis for promoting clinical application of PD-1/PD-L1 in AML.

The abnormal change in bone marrow microenvironment is also one of the reasons for inducing the deterioration of normal hematopoietic stem cells; the signal pathway in bone marrow microenvironment is also complex, involving a variety of regulatory factors [7]. As one of the regulatory factors in bone marrow microenvironment, soluble Ecadherin (SE-Cad) can reflect the invasion and metastasis ability of tumor cells [8]. Previous reports indicated that the expression level of E-cad on the surface of bone marrow mononuclear cells membrane in leukemia patients is decreased, while the level of SE-Cad in serum has increased significantly, suggesting that SE-Cad may participate in the development of leukemia [9]. The expression of SE-Cad in AML is one of the aspects discussed in this study. Invasion and metastasis, are process which involve the degradation of extracellular matrix, and are the main characteristics of malignant tumors. Type IV collagen is the main component of extracellular matrix and MMP-2 belongs to type IV collagenase of the MMP family. It has been confirmed that MMP-2 level has close relationship with the onset and advance of bladder cancer, endometrial cancer and other solid tumors [10]. Besides, the role of

MMP-2 in the occurrence and development of AML, a special type of malignant tumor, is also the focus of attention in recent years. Based on the above research, our present study explored the clinical significance of PD-1, MMP-2, SE-Cad expression in AML, so as to provide a reference for clinical treatment and prognosis evaluation of AML.

Materials and methods

Main reagents and instruments

FITC labeled-mouse anti human CD4 monoclonal antibody (mAb), FITC labeled-mouse anti human CD8 mAb, FITC labeled mouse anti-human PD-L1 mAb and homotype control antibody are all purchased from Beckman Coulter, USA. SE-Cad ELISA Kit was purchased from Shanghai biyuntian Biotechnology Co., Ltd (Shanghai, China). MMP-2 and GAPDH primers were purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd (Beijing, China). FACS Calibur flow cytometer was purchased from BD company (USA). Multiskan FC microplate reader was purchased from Thermo company (USA). MX3000P Real-time polymerase chain reaction (PCR) instrument was purchased from GENE company (USA). LabCycler PCR instrument was purchased from Sensoquest company (Germany).

Patients

Eighty-eight AML patients who were diagnosed and treated in our hospital from January 2014 to January 2016 were selected as our study subjects (the primary diagnosis group) and 88 healthy people were picked as the control group, in the same period for a prospective cohort study. There were 49 males and 39 females in the primary diagnosis group, with an average age of 41.4 ± 10.9 years. There were 51 males and 37 females, with an average age of 42.1 ± 10.2 years, in the control group. All AML patients received one standard chemotherapy session and the 88 AML patients were further separated into the remission group (37 cases) and the non-remission group (51 cases) according to Blood Disease Diagnosis and Curative Effect Standard issued by Zhang et al. in 2007 [11]. Informed consent form was received from all subjects, and the present study was authorized by the hospital ethics committee.

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The remission group included complete remission and partial remission: (1) For complete remission, the proportion of bone marrow primordial cells $\leq 5\%$; the absolute value of peripheral blood neutrophils $\geq 1.5 \times 10^9/L$; platelets $\geq 100 \times 10^9/L$; no extramedullary leukemia. (2) Partial remission: the proportion of bone marrow primordial cells decreased by more than 50%, about 0.05-0.25; other criteria were the same as complete remission. The non-remission group was not within the standard of complete and partial remission.

Patient selection

Inclusion criteria: (1) No previous myelodysplastic syndrome history. (2) The diagnosis and type of AML patients need to meet the relevant diagnostic criteria [1]. (3) Those who had not received radiotherapy and chemotherapy before. (4) Associated data are complete. (5) ECOG score is 0-2 [12].

Exclusion criteria: (1) Patients with severe infectious diseases. (2) Patients with organ dysfunction such as liver and kidney. (3) Patients with water electrolyte or acid-base imbalance. (4) Patients who have difficulty tolerating the chemotherapy program in this study. (5) Patients with hematopoiesis or other malignant blood diseases and blood tumors. (6) Patients with poor compliance or loss of visits.

Methods

Five mL of venous blood and 5 mL of bone marrow fluid from every patient were collected on an empty stomach early in the morning on the day after hospitalization. Five mL of venous blood and 5 mL of bone marrow fluid from the patients in remission group and non-remission group were collected on an empty stomach early in the morning one week after one course of chemotherapy.

Serum was separated by centrifuging the blood samples at 3000 R/min, for 15 min. After serum separation, the proportion of immature-bone marrow cells in the primary diagnosis group and the ratio of CD4⁺, CD8⁺ T cells, and PD-1 production on CD4⁺ and CD8⁺ T cells surfaces in peripheral blood of each group were detected by BD FACS Calibur flow cytometry. The average fluorescence intensity of PD-1 mAb and the same control antibody was obtained by Cellquest software to calculate the ratio of fluorescence intensity, which equals the

fluorescence intensity of PD-1/fluorescence intensity of the control antibody.

Bone marrow mononuclear cells were isolated by centrifuging the bone marrow fluid samples at 2000 R/min for 15 min. Then, the total mRNA of bone marrow mononuclear cells was extracted, and the expression rate and level of MMP-2 in bone marrow mononuclear cells were further detected by RT-PCR. The related primers were listed as following: MMP-2 (forward: 5'-TGACTTTCTTGGATCGGGTCG-3', reverse: 5'-AAGCACCACATCAGATGACTG-3'), GAPDH (forward: 5'-TGTGGGCATCAATGGATTTGG-3', reverse: 5'-ACACCATGTATTCCGGGTCAAT-3'). Reverse transcription is performed following the protocol of reverse transcription Kit. RT-PCR was conducted followed by the instructions of SYBR RT-PCR kit. The reaction system: pre-denatured at 95°C for 30 s, 95°C for 5 s, 60°C for 30 s, 35 cycles.

Relative gene expression of MMP-2 and GAPDH was quantified by the $2^{-\Delta\Delta Ct}$ method and GAPDH was used as the internal control. The experiment was replicated three times for the average value.

AML patients were followed up for two years, and their survival was recorded.

Statistical analysis

Statistical analysis was performed using SPSS22.0. The numeration data are presented as the frequency and percentage and the measurement data is showed as mean \pm SD. For the comparison between the two groups, t test was used for the measurement data, and χ^2 test was used for the comparison of the numeration data. The relationship between PD-1, MMP-2, SE-Cad and the proportion of bone marrow immature cells was analyzed using the Pearson correlation method. ROC curve was used to analyze the value of PD-1, MMP-2 and SE-Cad in evaluating the prognosis of patients. K-M survival curve was used to analyze the survival time (expressed as mean \pm SD) of different expression of each index. *P*-value < 0.05 was considered to be statistically significant.

Results

General data comparison of each group

The results showed that there was no significant difference regarding the age and gender

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Table 1. General data comparison of each group

Index	Primary diagnosis group (n=88)	Remission group (n=37)	Non-remission group (n=51)	Control group (n=88)	P value
Gender (cases)					>0.05
Male	49	20	28	51	
Female	39	17	23	37	
Age (year)	41.4±10.9	42.0±9.9	41.1±10.2	42.1±10.2	>0.05
BMI (kg/m ²)	22.94±2.31	22.71±2.28	23.08±2.25	22.86±2.42	>0.05
ALT (U/L)	27.31±4.42	29.58±4.27	28.39±4.53	27.00±4.47	>0.05
Platelet count (×10 ⁹ /L)	44.38±12.29	44.21±11.85	44.59±13.22	44.86±13.41	>0.05
Neutrophil count (×10 ⁹ /L)	0.84±0.27	0.82±0.34	0.89±0.36	0.81±0.42	>0.05
History of high myelodysplastic syndrome (cases)	36	14	22		>0.05
FAB typing (cases)					>0.05
M1	5	2	3		
M2	21	9	12		
M3	22	9	13		
M4	20	8	12		
M5	15	7	8		
M6	5	2	3		

Table 2. Comparison of CD4⁺, CD8⁺, CD4⁺/CD8⁺ ratio in peripheral blood of each group

Groups	CD4 ⁺	CD8 ⁺	CD4 ⁺ /CD8 ⁺
Primary diagnosis group (n=88)	30.24±8.74*	40.98±9.76*	0.82±0.43*
Remission group (n=37)	38.45±6.78#	32.97±8.21#	1.25±0.34#
Non-remission group (n=51)	34.53±7.87*#	36.54±7.22*#	0.99±0.36*#
The control group (n=88)	37.21±9.94	33.19±7.85	1.21±0.27

Note: *P<0.05 vs the control group, #P<0.05 vs the primary diagnosis group.

composition among groups (all P>0.05) (**Table 1**).

Comparison of CD4⁺, CD8⁺, CD4⁺/CD8⁺ ratio in peripheral blood among groups

The level of CD4⁺ and ratio of CD4⁺/CD8⁺ in peripheral blood of the primary diagnosis group was significantly lower than that of the control group (all P<0.05), while the level of CD8⁺ was clearly higher than that of the control group (P>0.05). Compared with the primary diagnosis group, the level of CD4⁺ and ratio of CD4⁺/CD8⁺ in the remission group and the non-remission group increased (all P<0.05), while the level of CD8⁺ decreased (P<0.05). Compared with the control group, no significant difference (P>0.05) in each index was shown in the remission group. While, the level of CD4⁺ and ratio of CD4⁺/CD8⁺ decreased (P<0.05), and the level of CD8⁺ was elevated in the non-remission group compared with the control group (P<0.05) (**Table 2**).

The results showed that the positive rate and level of PD-1 on CD4⁺ and CD8⁺ cells surfaces in primary diagnosis group were significantly higher than that in control group. Besides, the positive rate and level of PD-1 on the surface of CD4⁺ and CD8⁺ T cells in

and the non-remission group decreased compared with the primary diagnosis group (all P<0.05). The positive rate and level of every index in the remission group had no significant difference (P>0.05) compared with the control group, but the positive rate and level of PD-1 in the non-remission group increased as compared to control group (P<0.05) (**Table 3**).

Comparison of relative expression of MMP-2 mRNA in bone marrow mononuclear cells among groups

The positive rate and relative expression of MMP-2 mRNA in bone marrow mononuclear cells of the primary diagnosis group were significantly higher than the corresponding index in the control group. Besides, the positive rate and relative expression of MMP-2 mRNA of mononuclear cells in both the remission group and the non-remission group were down-regulated compared with the primary diagnosis group. In addition, compared with the control

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Table 3. Comparison of the ratio of PD-1 on the surface of CD4⁺ and CD8⁺ T cells among each group

Groups	PD-1+CD4 ⁺ T cells positive rate (%)	PD-1+CD4 ⁺ T cells (%)	PD-1+CD8 ⁺ T cells positive rate (%)	PD-1+CD8 ⁺ T cells (%)
Primary diagnosis group (n=88)	65 (73.86)*	19.17±8.24*	68 (77.27)*	17.29±7.77*
Remission group (n=37)	5 (13.51)#	5.91±1.98#	5 (13.51)#	6.16±2.32#
Non-remission group (n=51)	22 (43.14)*.#	16.88±2.10*.#	23 (45.10)*.#	14.96±1.60*.#
The control group (n=88)	12 (13.64)	5.44±1.33	13 (14.77)	5.59±2.17

Note: *P<0.05 vs the control group, #P<0.05 vs the primary diagnosis group.

Table 4. Comparison of relative expression of MMP-2 mRNA in bone marrow mononuclear cells among each group

Groups	Cases	MMP-2 mRNA positive rate	MMP-2 mRNA relative expression
Primary diagnosis group	88	63 (71.59)*	0.44±0.21*
Remission group	37	6 (16.22)#	0.14±0.019#
Non-remission group	51	23 (45.10)*.#	0.56±0.13*.#
Control group	88	13 (14.77)	0.13±0.056

Note: *P<0.05 vs the control group, #P<0.05 vs the primary diagnosis group.

Table 5. Comparison of serum SE-Cad levels among each group

Groups	Cases	SE-Cad positive rate	SE-Cad (ng/mL)
Primary diagnosis group	88	67 (76.14)*	69.66±25.52*
Remission group	37	6 (16.22)#	26.95±4.09#
Non-remission group	51	26 (50.98)*.#	56.15±19.32*.#
Control group	88	15 (17.05)	25.71±5.78

Note: *P<0.05 vs the control group, #P<0.05 vs the primary diagnosis group.

group, no significant difference was shown in the positive rate and relative expression of MMP-2 mRNA in the remission group (all P>0.05) but these indicators were both increased in the non-remission group (P<0.05) (**Table 4**).

Comparison of serum SE-Cad levels among each group

The results showed that the positive rate and level of serum SE-Cad in the primary diagnosis group were significantly higher than those in the control group (P<0.05). However, the positive rate and level of serum SE-Cad in the remission group and the non-remission group both decreased (P<0.05) compared with the primary diagnosis group. In addition, the positive rate and relative expression of serum SE-Cad in the remission group had no significant difference as compared to control group (P>0.05) but corresponding indices were increased in the non-remission group as com-

pared to control group (P<0.05) (**Table 5**).

Correlation between PD-1, MMP-2, SE-Cad and the proportion of bone marrow immature cells in the primary diagnosis group

The results showed that PD-1 on CD4⁺ cells surface, PD-1 on CD8⁺ cells surface, serum SE-Cad level and serum MMP-2 level were all positively associated with the proportion of bone marrow immature cells (r=0.875, 0.504, 0.683, 0.680; and P=0.000, 0.000, 0.000, 0.000, respectively) (**Figure 1**).

Results of ROC curve

The results showed that 37 patients survived and 51 died after 2 years of follow-up. The survival rate was 42.05%. The level of PD-1 on CD4⁺ cells and CD8⁺ cells surface, serum SE-Cad level and serum MMP-2 level in the primary diagnosis group all had a high predictive value in evaluating the prognosis of AML patients (**Figure 2** and **Table 6**).

Results of K-M survival curve

M survival analysis suggested that the short-term survival time of patients who had a negative index including PD-1 on CD4⁺ cells and CD8⁺ cells surface, serum MMP-2 and serum SE-Cad was significantly longer than that of patients with a positive index (P<0.05) (**Figure 3** and **Table 7**).

Discussion

Immune dysfunction is closely related to the onset and progress of AML. In clinical practice, the detection of T-lymphocyte subsets is often

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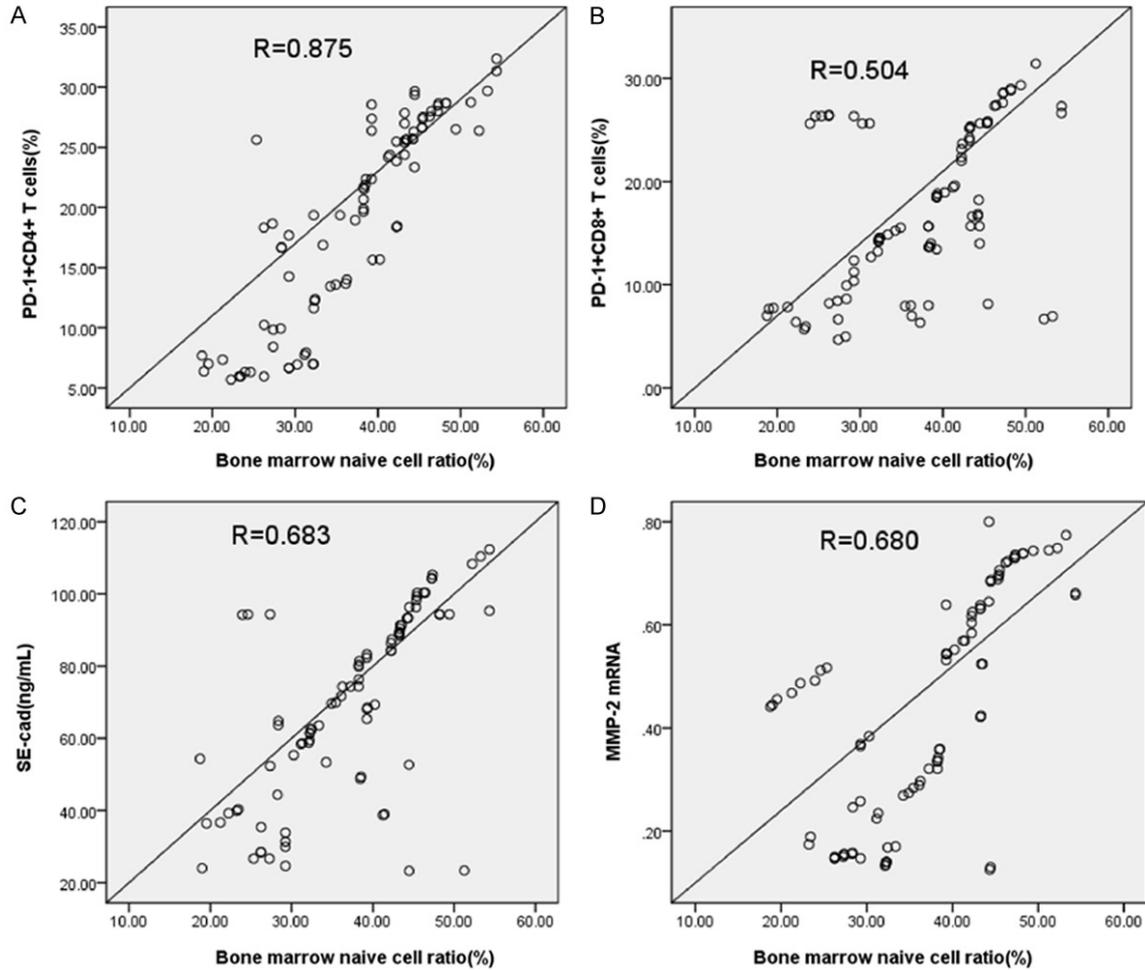


Figure 1. Perason correlation analysis. A: Correlation between PD-1 on the surface of CD4⁺ cells and the proportion of bone marrow immature cells in the primary diagnosis group. B: Correlation between PD-1 on the surface of CD8⁺ cells and the proportion of bone marrow immature cells in the primary diagnosis group. C: Correlation between serum SE-Cad level and the proportion of bone marrow immature cells in the primary diagnosis group. D: Correlation between serum MMP-2 level and the proportion of bone marrow immature cells in the primary diagnosis group.

used as one of the indicators to evaluate the immune function of AML patients [13]. Our present study detected abnormal proportion of CD4⁺ and CD8⁺ cells in the primary diagnosis group. However, the proportion of CD4⁺, CD8⁺ and CD4⁺/CD8⁺ in both the remission group and the non-remission group were all improved after treatment. Although the detection of T-lymphocyte subsets is widely used in clinical practice, it is difficult to simply reflect upon the treatment and prognosis of patients through the level of T-lymphocyte subsets because the level of change of such indicators in the body is often affected by multiple factors [14]. Thus, it is the focus of clinical researchers to explore specific indicators to evaluate the patients' condition, treatment and prognosis.

With the development of medical Science, more and more researchers explain the immune escape mechanism through immune checkpoints [15]. PD-1/PD-L1, as one of the immune checkpoints, can inhibit the signal transduction of T cell receptors and down-regulate the expression of anti-apoptotic molecules and pro-inflammatory factors [16]. Besides, the PD-1/PD-L1 complex has a certain effect on cell cycle, so as to promote the immune cells escape from the killing effect of immune system, and thus let tumor cells continuously proliferate in the body [17, 18]. A study reported that the level of PD-1 on the surface of a T-lymphocyte subtype was over expressed, measured by detecting the peripheral blood and bone marrow of patients who were newly

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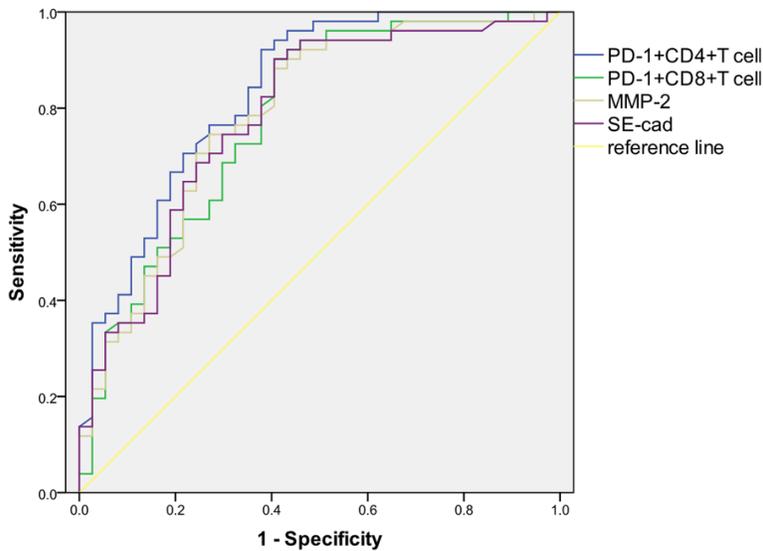


Figure 2. Results of ROC curve.

diagnosed and relapsed AML [19]. Some other studies demonstrated that the higher the level of PD-1, the worse the prognosis of AML patients [20]. The results of our present study show that the level of PD-1 on CD4⁺ and CD8⁺ T cells surface in the primary diagnosis group increased significantly as compared to the control group. Then, the level of PD-1 in the remission group and non-remission group both decreased after treatment, but the level of PD-1 in the non-remission group was still higher than that of the control group. The results of correlation analysis showed that the levels of PD-1 on the surface of CD4⁺ and CD8⁺ cells were positively associated with the proportion of immature bone marrow cells ($r=0.875$, 0.504 , respectively), and the ROC results exhibited that the AUC of PD-1 on the surface of CD4⁺ and CD8⁺ was 0.834 and 0.780 , respectively. In line with the above results, the K-M survival analysis displayed that the survival time of PD-1 negative patients was much higher than that of PD-1 positive patients after the two-year follow-up. The above results suggest that PD-1 is closely involved in the onset of AML and has a certain clinical value in evaluating the prognosis of patients, providing a theoretical basis for PD-1 targeted therapy.

MMPs exert an important role in the bone marrow microenvironment. It was found that MMPs can regulate the growth, proliferation, differentiation, adhesion and migration of many kinds

of cells by regulating the structure of extracellular matrix in the bone marrow of normal people. MMP-2 is a type IV collagenase in the MMPs family, which is mainly secreted in the form of collagen [21]. Under normal physiological conditions, the body does not secrete a small amount of MMP-2. However, it is discovered that MMP-2 is relatively highly expressed in acute leukemia (AL), and the positive rate of MMP-2 is even 65% in adult AL [22]. In this study, we found that relative expression level of MMP-2 mRNA in the primary diagnosis group was significantly elevated compared with that of the control group.

Subsequently, positive rate and relative MMP-2 mRNA expression in the remission group and the non-remission group were both suppressed after treatment, but the level of MMP-2 mRNA in the non-remission group was still higher compared with the control group. Besides, positive correlation exists between MMP-2 and the proportion of immature bone marrow cells in the primary diagnosis group ($r=0.680$), and the AUC of MMP-2 in the prognosis of AML patients was 0.786 . What is more, the results of K-M survival analysis showed that the patients with negative MMP-2 expression had a significantly longer survival time than the patients with positive MMP-2 expression.

SE-Cad and membrane-based E-cad are two main forms of E-cad in organisms. The decrease of adhesion between tumor cells is closely related to the onset and progress of the tumor [23, 24]. By down-regulating the adhesion between hematopoietic cells and stroma, membrane-based E-cad acts as an important regulator in the development of leukemia and promotes immature cells to enter the blood. However, when the membrane-based E-cad falls off and enters into the blood circulation, it will degrade to SE-Cad, which further lead to a significant increase of SE-Cad level in the blood [25, 26]. Our research revealed that serum SE-Cad level in the primary diagnosis group was elevated compared with the control group, and then the level of SE-Cad in the remission

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Table 6. Results of ROC curve

Index	Cut-off value	AUC	Standard error	P value	95% CI	Sensitivity (%)	Specificity (%)
PD-1+CD4 ⁺ T cell (%)	13.84	0.834	0.044	0.000	0.748, 0.921	92.20	62.20
PD-1+CD8 ⁺ T cell (%)	13.34	0.780	0.051	0.000	0.680, 0.880	90.20	59.50
MMP-2	0.293	0.786	0.05	0.000	0.687, 0.884	88.20	59.50
SE-Cad (ng/mL)	56.86	0.784	0.051	0.000	0.684, 0.883	90.20	59.50

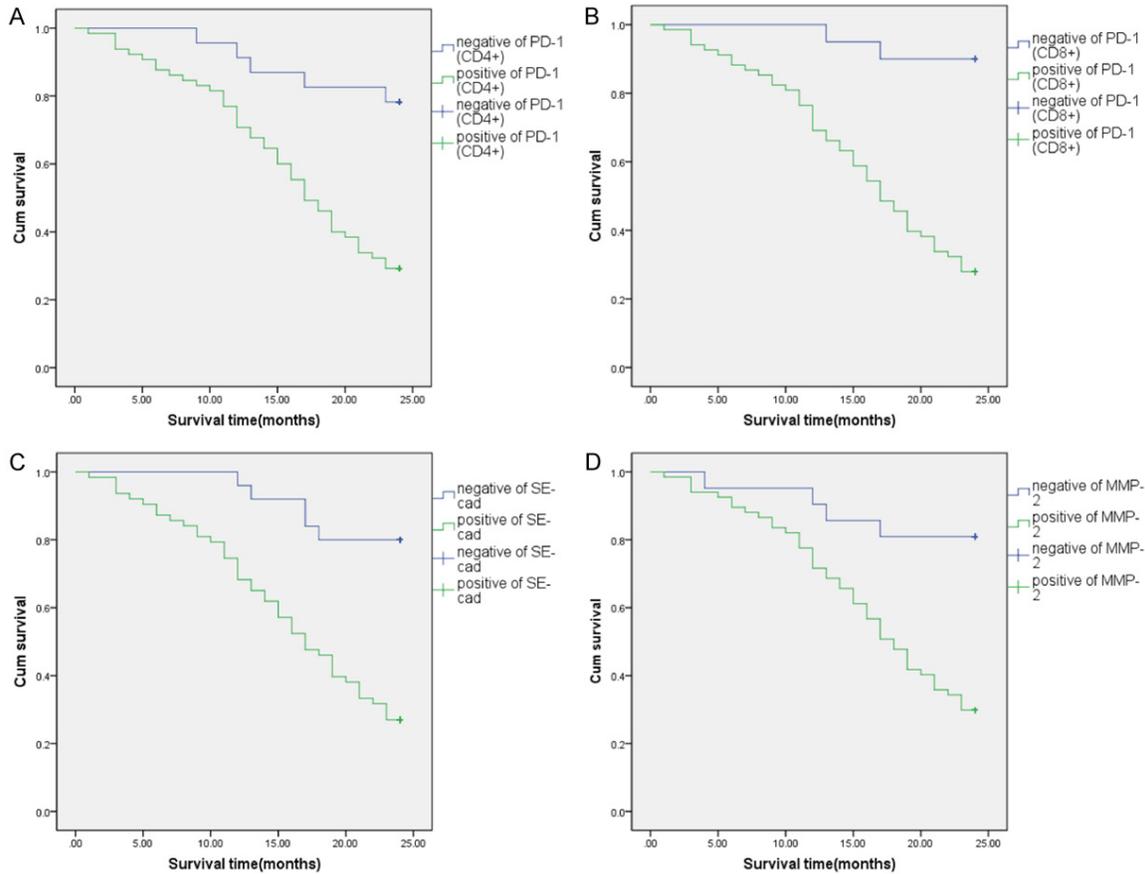


Figure 3. Results of K-M survival curve. A: Survival curve of positive and negative PD-1 on the surface of CD4⁺ cells in the primary diagnosis group. B: Survival curve of positive and negative PD-1 on the surface of CD8⁺ cells in the primary diagnosis group. C: Survival curve of positive and negative serum SE-Cad level in the primary diagnosis group. D: Survival curve of positive and negative serum MMP-2 level in the primary diagnosis group.

Table 7. Comparison of survival time of different expression of each index

Groups	Mean survival time (month)	95% CI
Negative PD-1 on the surface of CD4 ⁺ cells*	22.0±0.9	20.2, 23.8
Positive PD-1 on the surface of CD4 ⁺ cells	16.6±0.9	15.0, 18.3
Negative PD-1 on the surface of CD8 ⁺ cells*	23.1±0.6	21.9, 24.3
Positive PD-1 on the surface of CD8 ⁺ cells	16.5±0.8	14.9, 18.2
Negative MMP-2 in bone marrow mononuclear cells in the primary diagnosis group*	22.3±0.7	20.9, 23.7
Positive MMP-2 in bone marrow mononuclear cells in the primary diagnosis group	16.3±0.9	14.6, 18.1
Negative serum SE-Cad in the primary diagnosis group*	21.7±1.2	19.3, 23.9
Positive serum SE-Cad in the primary diagnosis group	16.9±0.8	15.3, 18.5

Note: *P<0.05 vs positive group with the same index.

group and the non-remission group decreased but after treatment, but it was still higher in the non-remission group than in the control group. Besides, positive correlation between SE-Cad and the proportion of bone marrow immature cells in the primary diagnosis group was discovered ($r=0.683$), and the AUC of SE-Cad was 0.784. Moreover, K-M survival analysis showed that the survival time of the patients who had negative SE-Cad expression was significantly longer than that of the patients who had positive SE-Cad expression, also indicating that SE-Cad is closely related to the onset of AML and it has certain clinical value in prognosis evaluation.

However, there still exist limitations in the study including small sample capacity, and it lacks related analysis about the differences among various indicators in different types of FAB patients. So our results still need further follow-up study to confirm.

In conclusion, PD-1, MMP-2 and SE-Cad levels in AML patients are highly expressed, and they are all positively associated with the proportion of immature bone marrow cells, suggesting that PD-1, MMP-2 and SE-Cad may participate in the occurrence of AML and providing relevant basis for AML treatment and prognosis.

Acknowledgements

This work was supported by the Science and Technology Project of the Health and Family Planning of Lianyungang for Clinical study on the causes of drug resistance or intolerance of imatinib in CML patients and the timing of dressing change (201711).

Disclosure of conflict of interest

None.

Address correspondence to: Ying Wang, Department of Hematology, The First People's Hospital of Lianyungang, No.182 Tongguan North Road, Haizhou District, Lianyungang 222000, Jiangsu Province, China. Tel: +86-0518-85605042; Fax: +86-0518-85605042; E-mail: wangying2wy5@163.com

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