Int J Clin Exp Med 2014;7(10):3714-3718
www.ijcem.com /ISSN:1940-5901/IJCEM0001963

Original Article

Immuno-phenotypes and prognosis of acute leukemia in elderly patients

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Received August 18, 2014; Accepted September 20, 2014; Epub October 15, 2014; Published October 30, 2014

Abstract: Objective: This study is to provide reliable experimental treatment options for the diagnosis of acute leukemia, prognosis analysis and the detection of minimal residual disease. We observed the bone marrow CD123/CD117/CD34/HLA-DR antigen expression in 64 elderly patients with acute leukemia (AL). Methods: The immune phenotypes of 64 elderly AL patients were detected and the correlations of CD123, HLA-DR, CD34 and CD117 expression with the leukemia cell morphology were analyzed. The cell genetics, molecular biology and the prognostic stratification were compared based on flow cytometry. Results: In CDI23-positive patients, the complete remission (CR) rate was 21.5%. In CDI23-negative patients, the CR rate was 59.1%. The CR rate was 21.9% in HLA-DR-positive patients and 43.8% in HLA-DR-negative patients. The CR rate was 24.0% in CD34-positive patients and 41.1% in CD34-negative patients. The CR rate was 29.0% in CD117-positive patients and 42.3% in CD117-negative patients. The CR rate was 34.4% in CD38-positive patients and 0.00% in CD38-negative patients. Conclusions: These results suggest that $CD123^+$, $CD117^+$, $CD34^+$, and $HLA\text{-}DR^+$ are the factors related with the poor prognosis of elderly patients with acute leukemia.

Keywords: Acute leukemia, CD123, elderly patients, flow cytometry

Introduction

CD123 molecule is encoded by interleukin-3 receptor (IL-3R) as one family member of hematopoietic growth factor receptors, which expressed in undifferentiated leukemia cells [1]. IL-3R can be widely expressed in the blood malignancies [2]. Activation of cytokine receptor affects the hematopoietic cell survival, proliferation and differentiation. Expression of tyrosine kinase receptor (c-kit) and fms-like tyrosine kinase-3 (FLT3) can be found in the cells with CDI23 antigen [3, 4]. In recent years, tumorigenicity of IL-3R gene has been studied in animal models [5, 6]. The subsets of leukemia cells with $CD34^+CD38^-CDI23^+$ markers can cause leukemia after transplanted into animal model, indicating that these cells have the characteristics of leukemia stem cells and $CD34^+CD38^-CDI23^+$ can be used as surface marker of leukemia stem cells [7, 8]. Harald et al. [4] found $CD34^+/CD38^-$ cells carrying BCR/ABL gene highly expressed CD123 in chronic myeloid leukemia. Miroslav et al. [9] reported that the expression of CD123 in acute lymphoblastic leukemia cells of children is about 10% but CD123 is over-expressed in acute lymphoblastic leukemia cells of adult B-cell [10]. CD123 is considered a sign of leukemia stem cells. Hosen et al. [11] found that $CD34^+CD38^-CD96^+$ leukemia cells can also grow in immune-deficient mice so CD96 is also one of leukemic stem cell surface markers [12].

In this study, the immune phenotypes and their relationship with prognosis were analyzed in 64 cases of elderly patients with acute leukemia. The pathogenesis of acute leukemia in elderly patients and therapeutic approach were discussed. This study provides a scientific basis to improve clinical outcomes and prolong disease-free survival [13].

Materials and methods

Patients and samples

In this study, 64 elderly patients with AL in Yan'an University Hospital were enrolled in this

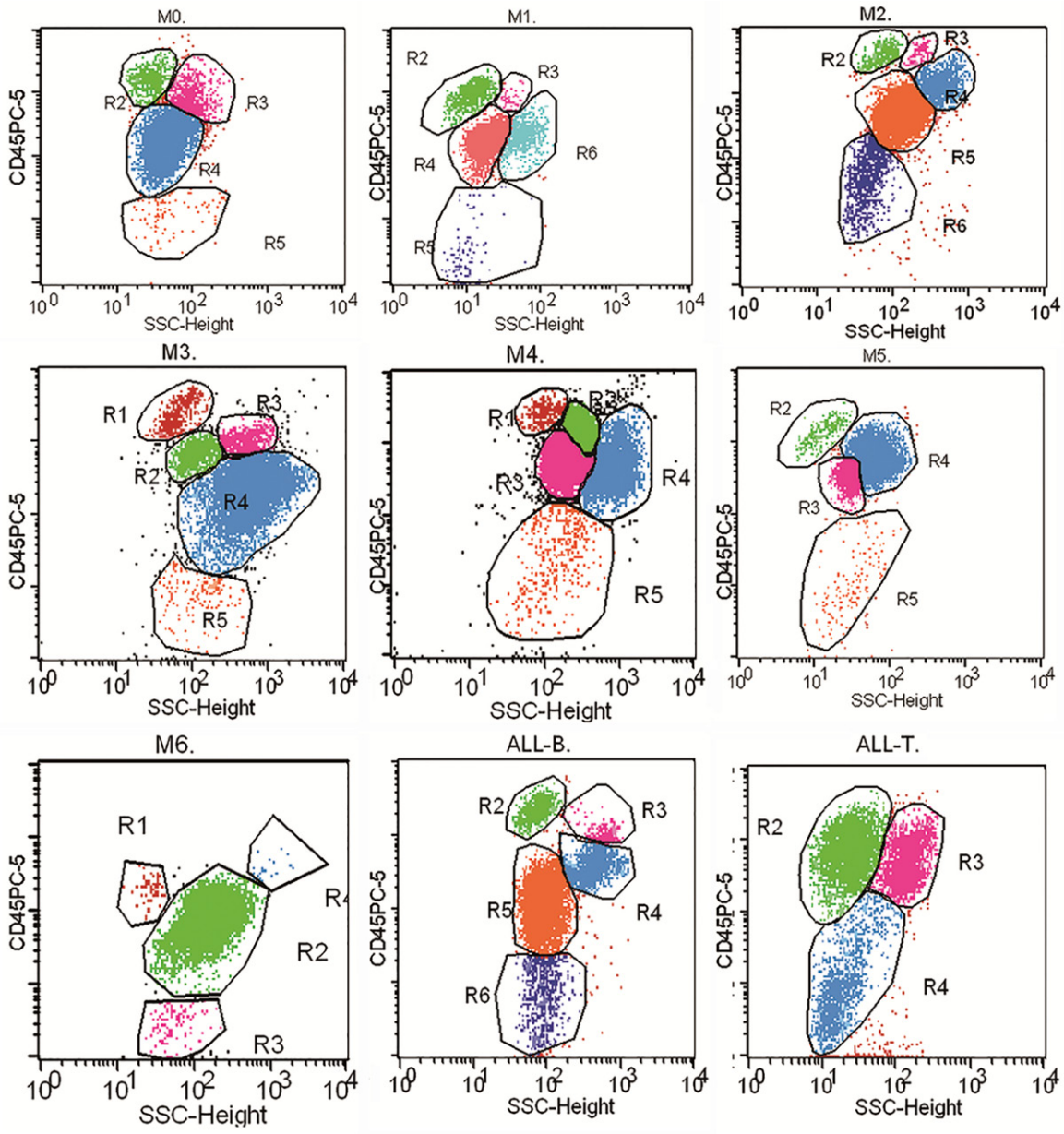

Figure 1. CD45/SSC gating diagram of different immunophenotypes in elderly patients with acute leukemia. Flow cytometry was performed to determine the immunophenotypes of the bone marrow cells in elderly patients with acute leukemia. The scattering angle (SSC-Height) and CD45 fluorescence intensity (CD45 PC-5) were used as parameters to determine the different cell populations, including lymphocyte populations, monocytes, promyelocytic cells, leukemia cells, nucleated red blood cells, and other groups. This experimental results can determine the source of leukemia cells.

hospital from 2008 to 2012. The median age of patients (35 men, 29 women) was 69 years (range, 60-82 years). 55 cases have acute myeloid leukemia (AML). 32 cases have increased peripheral leukocytes and 9 cases have acute lymphoblastic leukemia. Patients were selected based on clinical symptoms, general blood test, bone marrow morphologic test, immunephenotypes and genetic subtypes. AML cells with myeloid antigen and one or more lymphoid antigen (positive rate ≥ 30%) were Ly+AML. The patients without any lymphoid antigen were Ly-AML. Immunological diagnostic criteria of hybrid leukemia were based on The European Group for the Immunological Classification of Leukemia (EGIL) integration standards.

Table 1. Expression of $CD123^+$, $CD117^+$, $CD34^+$, $HLA\text{-}DR^+$, $CD38^+$ antigens in patients with various subtypes of acute leukemias

Groups	Numbers	$CDI23^+$	$HLA\text{-}DR^+$	$CD34^+$	$CD117^+$	$CD38^+$
M_0	1	1	0	1	1	1
M_1	8	7	6	7	8	8
M_2	22	11	10	9	14	22
M_3	10	4	0	1	5	10
M_4	7	5	6	0	7	7
M_5	6	6	2	4	4	6
M_6	1	1	1	0	0	1
ALL-B	7	5	6	2	0	7
ALL-T	2	2	1	1	1	2

Reagents

Monoclonal antibodies of CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD22, CD25, CD13, CD14 and CD45 were purchased from bdbiosciences (BD, USA). CD23, CD15, CD33, CD34, CD38, CD61, HLA-DR, Gly-A, MPO, CD79a, CD117 and CD123 antibodies were purchased from BECKMAN COULTER (USA). Isotype control of $CD45/IgG_1/IgG_1$ and red cell lysate were purchased from BD. All antibodies are labeled with FITC, PE and PerCP respectively.

Flow cytometry

Mononuclear cells from 2 ml heparinized bone marrow aspirates were prepared and 50 μL bone marrow aspirates aliquot were added to test tubes. Monoclonal antibodies used for bone marrow series were CD13, CD14, CD33, CD64 and CD117. B series were CD10, CD19, CD20, and CD22. T series were CD2, CD3, CD4, CD5, CD7, and CD8. The stem cell monoclonal antibodies were CD34, HLA-DR and CD123. Intracytoplasmic myeloperoxidase (MPO) and cCD3 were fixed and labeled. Each tube was added with heparin marrow 1×10^6/ml, were added to 20 μl tricolor directly labeled fluorescent antibodies. The samples were analyzed with FAC-Scalibur (BD, USA) flow cytometry with Cell-Quest software. For each tube, 10,000 cells were tested by using forward scattering angle (FSC) and side scattering angle (SSC) gating to exclude debris and dead cells. Using CD45/SSC (lateral angle scattering) gating, antigen expression of naive cell populations was calculated. Over 20% expression on the cell surfaces was considered as positive.

Remission induction therapy

The patients with acute promyelocytic cells were treated with all-trans retinoic acid and arsenic trioxide 10 mg/d×28 d. The patients with myeloid leukemia were treated with MA programs (mitoxantrone 4-6 mg/d×3 d, cytarabine 100-200 mg/d×5-7 d), DA program (daunorubicin 40 mg/d×3 d, cytarabine 100-200 mg/d×5-7 d), DAE program (homoharringtonine base 3 mg/d×5 d, cytarabine 100 mg/d×5 d, etoposide 100 mg/d×5 d), EA program (etoposide 100 mg/d×3 d, cytarabine 100-200 mg/d×5-7 d), a small dose cytarabine (25-50 mg/d×10-14 d). The patients with acute lymphoblastic leukemia were treated with VDLP scheme (vincristine 2 mg/d 1 d, 8 d, 15 d, 22 d, daunorubicin 40 mg/d 1-3 d, 15-17 d, asparaginase 10,000 units/d, every other day, a total of five times, prednisone 1 mg/kg/d 1-28 d).

Statistical analysis

Data were recorded as mean±standard deviation (SD). The obtained data are using SPSS11.5 software package for statistical analysis. Data were compared using ANOVA between multiple sets. $P < 0.05$ was considered statistically significant.

Results

Immunophenotyping gating diagram of elderly patients with acute leukemia

To determine immunophenotyping gating diagram of elderly patients with acute leukemia, flow cytometry was performed. Immunophenotyping gating diagram of elderly patients with acute leukemia was shown in **Figure 1**. A-I were CD45/SSC gating diagram of M0, M1, M2, M3, M4EO, M5, M6, ALL-B, and ALL-T patients. Immune phenotypes of 64 elderly patients with acute leukemia were shown in **Table 1**. Among them, 42 patients were CD123 positive with a rate of 65.7%. 32 patients were HLA-DR positive with a rate of 50.0%. 25 patients with CD34 positive had a rate of 39.1%. 38 patients with CD117 positive had a rate of 59.4%. 36 cases of $CD123^+$ $CD117^+$, 18 cases of $CD123^+$ $CD34^+$, 14 cases of $CD123^+$ $CD34^+$ $HLA\text{-}DR^+$, 10 cases of $CD123^+CD117^+CD34^+$, and 6 cases of $CD123^+CD117^+CD34^+$ $HLA\text{-}DR^+$ were detected.

Table 2. The relationship of acute leukemia phenotype with prognosis of patients who achieved two times of conventional chemotherapy

Immunophenotypes	Antigen positive		Antigen negative	
	Total No. of patients	No. of CR	Total No. of patients	No. of CR
CDI23	42	9	22	13
HLA-DR	32	8	32	14
CD34	25	5	39	16
CD117	40	12	24	10
CD38	64	22	0	0

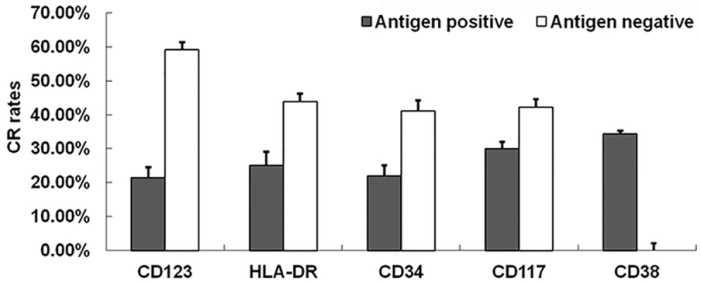

Figure 2. The relationship of immunophenotypes and prognosis. The relationship of acute leukemia phenotype with prognosis was analyzed. Twenty-two patients achieved CR with a rate of 4% after two times of conventional chemotherapy. The relationship of acute leukemia phenotypes and prognosis were indicated in the Figure.

Expression of CD123, HLA-DR, CD34, CD117, CD38 in 64 elderly patients with acute leukemia

As given in **Table 2**, 42 cases were CDI23 positive with a rate of 65.7%. The mean expression rate of CD123 in 55 cases of myeloid leukemia was 63.7%, in which the expression rate of CD123 was 96.9% in 32 patients with high leukocytes. 6 patients with acute promyelocytic leukemia are $CD123^-$ but the other 4 patients were $CD123^+$. 7 patients were CDI23 positive (expression rate 77.8%) in 9 patients with acute lymphoblastic leukemia. The rate of CDI23 was 100% in 5 patients with high leukocytes.

As given in **Table 2**, 32 cases were HLA-DR positive (50%). The average HLA-DR expression was 47.3% in patients with acute myeloid leukemia. Only one patient with high leukocytes was HLA-DR positive. The average HLA-DR expression rate was 77.8% in patients with acute lymphoblastic leukemia.

As given in **Table 2**, 25 cases were CD34 positive with an average rate of 39.1%, in which the average expression rate in patients with myeloid leukemia was 33.4%. Only one case had high leukocytes in M_3. The CD34 expression rate was 33.4% in patients with acute lymphoblastic leukemia.

As given in **Table 2**, 40 cases were CD117 positive with an average rate of 56.3%, in which the average expression rate in patients with myeloid leukemia was 63.7%. Only one case had high leukocytes in M_3. The CD117 expression rate was 11.2% in patients with acute lymphoblastic leukemia.

The relationship of acute leukemia phenotype with prognosis

The relationship of acute leukemia phenotype with prognosis was analyzed. Twenty-two patients achieved complete remission (CR) with a rate of 4% after two times of conventional chemotherapy. As shown in **Figure 2**, In CDI23-positive patients, the CR rate was 21.5%. In CDI23-negative patients, the CR rate was 59.1%. The CR rate was 21.9% in HLA-DR-positive patients and 43.8% in HLA-DR-negative patients. The CR rate was 24.0% in CD34-positive patients and 41.1% in CD34-negative patients. The CR rate was 29.0% in CD117-positive patients and 42.3% in CD117-negative patients. The CR rate was 34.4% in CD38-positive patients and 0.00% in CD38-negative patients. These results suggest that $CD123^+$, $CD117^+$, $CD34^+$, and $HLA\text{-}DR^+$ are the factors related with the poor prognosis of elderly patients with acute leukemia.

Discussion

In this study, the CD123 positive rate was 65.7%. The HLA-DR positive rate was 50.0% and the CD117 positive rate was 59.4%. CD123 expression showed the highest rate even higher than Zhu et al. report (33.7%) [3]. The CD123 expression is highly correlated with high leukocytes. The CD123 expression rate was high in B cells of elderly patients with acute lymphoblastic leukemia [2]. It was significantly higher than that expression in B cells of children but lower than that expression in B cells of adults with acute lymphocytic leukemia when compared

with Miroslav et al. report [9]. Generally, CD34 and HLA-DR are not expressed in patients with acute promyelocytic leukemia but expressed in patients with high leukocyte expression [13]. The CD117 is mainly expressed in patients with acute myeloid leukemia and the expression is low in patients with acute lymphoblastic leukemia. The CR rate is much lower in patients with CD123, HLA-DR, CD34 and CD117 antigen-positive expression than negative ones. High expression of CD123, HLA-DR, CD34 and CD117 antigen could be a high negative factor for prognosis.

Disclosure of conflict of interest

None.

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