Clinical Characteristics and Prognosis of 167 Cases of Acute Erythroleukemia

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Abstract To observe the biological characteristic and the prognoses in patients with acute erythroleukemia (AEL). The results of 167 patients with newly diagnosed AEL, from January 2004 and June 2014 in the department of Hematology, Shandong Province Chinese Medicine Hospital, were reviewed by morphology, immunology, cytogenetics, molecular biology. Flow cytometry analysis indicated that CD13 (96.1 %), CD33 (95.1 %), CD117 (87.4 %) and CD34 (79.4 %) were highly expressed in AEL. 56 of 148 (37.8 %) AEL patients had a variety of cytogenetic abnormalities, 27 of 148 (18.2 %) patients were complex karyotype (abnormal involving 3 or more chromosomes), the abnormalities of chromosomes 3, 5, 7 and 8 were more frequently involved and the most common one was +8, accounting for 35.7 % of all abnormal karyotype, followed by 5q- (17.9 %). Mutation analysis showed CEBPA mutation ratio of AEL patients was 44.0 % (11/25), that of NPM1as 15.4 % (4/26). Initial induced remission rate of AEL was 56.6 % (30/53), compared by 33.3 % (4/12) of MDSM6. Survival analysis was shown that the overall survival in female was better than that in male ($P = 0.047$). The overall survival time of transplantation group was significantly longer than chemotherapy group ($P = 0.000$). The OS of 13–39 years old group was the best, 40–49 years old group took second place, >50 years old group appeared to be the worst. Patients with AML-M₆

had dysplasia in varying degrees in granulocyte, erythrocyte and megakaryocyte series. Periodic acid—Shiff reaction staining in polychromatic erythroblast and orthochromatic erythroblast had a specificity in the diagnosis of AEL. AEL had its own unique biological features, and allogeneic hematopoietic stem cell transplantation could significantly improve its poor prognosis.

Keywords Leukemia · Erythroblastic, acute · Disease attributes · Prognosis

Introduction

Acute erythroleukemia (AEL) is a subtype of acute myeloid leukemia (AML) [1]. The disease is common in adults, the prognosis is poor, clinical manifestations include stubborn anemia with irregular fever, late liver splenomegaly, skin purpura, repeated infection, and stomatitis, bone pain and Sternal tenderness, rare lymph node enlargement. In 1976, the FAB cooperative group has defined the erythroleukemia (EL) as the AML-M₆ [2]. In 1985, The diagnostic criteria were revised as [3]: nucleated red blood cells in bone marrow ≥ 50 %, the original granulocytes or monocytes (I + α type) ≥ 30 %, (except for the plasma cells, lymphocytes, mast cells, macrophages, and nucleated red blood cells).

Previous reports show that the AEL chemotherapy remission rate is low, high recurrence rate and survival period is short [4, 5]. Because of the complicated form AEL, relatively insensitive to chemotherapy, and poor prognosis, which prompted people to explore the potential reason, so the comprehensive research of the relationship between the biological characteristics and prognosis of AEL is necessary.

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Patients and Methods

Between January 2004 and June 2014, the hospital received 4100 AML patients, including 167 patients with AEL (4.1 %) were enrolled in the study. 167 patients meet the 2008 WHO classification criteria for the diagnosis of hematopoietic tissue tumors. Inclusion criteria: Patients older than 18 years old. Patients were non-initial bone marrow metastasis. Patients with normal kidney, liver and lung function.

Cell Morphology

Patient bone marrow smears after Wright's staining for cell morphology. Some smears do additional cytochemistry staining, including bone marrow peroxidase (POX), non-specific esterase naphthol acetate esterase (NAE) and sodium fluoride inhibition test, periodic acid–Schiff (PAS) staining.

Karyotype Analysis

Extraction of bone marrow in patients with 5–6 ml, heparin anticoagulation, 24 h training specimens (preparation of chromosome suspension), perform karyotype analysis after R banding. Abnormal chromosome karyotype described according to the human genetics international naming system (ISCN2005).

Leukemia Immunophenotyping

Application of flow cytometry indirect immunofluorescence test leukemia cell surface antigen. Adopted antibodies, including myeloid surface marks: (anti-CD13, anti-CD33, anti-CD11b, anti-CD117, anti-MPO, anti-CD14, CD15 monoclonal antibody), lymphoid surface antibodies (anti-CD2, anti-CD3, anti-CD7, anti-cCD3, anti-CD10, anti-CD19, anti-CD20 and cCD79a antibody) and non-specific antibodies (anti-HLA-DR, anti-CD34, anti-CD38 and CD56 monoclonal antibody). Judging criteria: cytoplasm antigens ≥ 10 % positive, cell membrane surface antigen ≥ 20 % positive.

Molecular Biological Detection

Using multiple polymerase chain reaction (PCR) to detect 62 cases of patients with AEL 29 kinds of common fusion gene transcription, including the BCR-ABL, AML-ETO, CBF β -MYH11, PML-RAR α , MLL-MLL, DEK-NUP214. There are enough DNA samples of 20 patients with AML detect common gene mutations, including NPM1 (12th exon), CEBPA (TAD1, TAD2, bZIP), FLT3-ITD (exon

15), FLT3-TKD (20th exon), c-Kit outer (8th and 17th exon) and DNMT3A gene.

Treatment and Follow-Up

Cases of induction therapy with domestic routine chemotherapy regimens, standard (3 + 7) combination chemotherapy, IA scheme (idarubicin 10 mg/m², application of 3 days, cytarabine 100 mg/m², application of 7 days. 23 patients received allogeneic hematopoietic stem cell transplantation, Pretreatment scheme for modified BUCY [semustine (Me-CCNU) 250 g m²/day, hydroxyurea (HU) 40 mg/kg each time, 2 times. Cytarabine (Ara-C) 2 g/m², Busulfan (BU) 4 mg kg per day, stay for 3 days, cyclophosphamide (CTX), 1.8 g/m², every day for 2 days. Follow-up ended in August 2013, the time of disease diagnosed until death or follow-up end time as overall survival (OS).

Statistical Analysis

The Chi-square test was used for categorical data, the comparison between the measured data using Wilcoxon two independent samples nonparametric tests. Kaplan–Meier method to analyze OS and survival curve, The Log-rank test was used for survival time difference between the two groups. IBM SPSS (Statistical Package for the Social Sciences) version 19.0 (IBM Corp. Armonk, NY, USA) was used for the statistical analysis. The difference was statistically significant ($P < 0.05$).

Results

General Characteristics

167 AEL patients with fatigue symptoms followed by skin and mucous membrane bleeding and fever before diagnosis. Male 97 and female 70, the gender ratio was 1.39:1, the median age of 51 years old (13–83). Initial diagnosis, 35.6 % of patients had symptoms of skin and mucous membrane bleeding, 33.7 % of patients have fever. In peripheral blood, the median WBC was 2.6 (0.5–179.4) $\times 10^9$ /l, the median HGB was 75 (36–231) g/l, the median PLT was 38 (10–321) $\times 10^9$ /l.

Cell Morphology and the Immune Phenotype

All cases of bone marrow primitive cells accounted for 20 % or more of non-erythroid nucleated cells (NEC), the RBC system accounted for 50–80 % of the ANC. No dysplasia on bone marrow morphology was observed.

In addition, bone marrow smear tests found that red blood cell accounted for all bone marrow nucleated cells (ANC) median percentage of 60.0 % (50.0–97.5 %), Bone marrow primitive cells accounted of ANC a median percentage was 16.0 % (0.5–89.5 %). Red blood pathological hematopoietic in 76.6 % of patients, 19.8 and 18.0 % of patients with grain, giant nuclear pathological hematopoietic respectively.

Primitive bone marrow cells took up of the ANC median was 16.0 % (0.5–43.5 %), took up of the NEC median percentage was 40.5 % (20.0–93.5 %). 149 patients underwent immune phenotype analysis at the same time. The myeloid antigen expression in 131 cases, 12 cases of myeloid were mononuclear cell antigen expression, 3 cases of myeloid were red blood cell antigen expression, 3 cases of myeloid were lymphoid antigen expression (Table 1).

Cytogenetic Characteristics

148 patients success to begin with chromosome karyotype analysis, with 92 cases of normal karyotype (62.2 %), abnormal karyotype 56 cases (37.8 %); Complex karyotype (three or more chromosome abnormality) 27 cases (18.2 %).

Molecular Biology Characteristics

3 cases of MLL–MLL tandem repeats transcription were detected, 2 cases with normal karyotype, 1 case of abnormal karyotype (46, XY, 13 q-[3]/46, XY [7]); 2 cases of BCR-ABL fusion gene was detected, The karyotype were 46, XX, t (9; 22; 13) (q34; q1 1; q22), 21 q + [6]; and 46, XX, t (9; 22) [9]/46, idem, t (4; 19) (q13; p13) [1], respectively (Table 2).

Table 1 149 cases of patients with acute erythroleukemia immune phenotype

Antigen	Number of patients	Number of positive (%)
CD13	149	72(48.3)
CD33	149	67(45.0)
CD117	149	77(51.6)
HLA-DR	149	74(49.7)
GPA	149	60(40.2)
CD71	149	56(37.6)
CD15	149	36(24.2)
MPO	149	17(11.4)

Table 2 149 cases of common gene mutations in patients with acute red leukemia

Gene	Detection no.	Mutation no.	Mutation rate (%)
NPM1	26	4	15.4
CEBPA	25	11	44.0
FLT3	24	2	8.3
C-Kit	25	0	0
DNMT3A	13	0	0

Treatment

(1) Routine chemotherapy regimens: 53 patients (male 29 cases, female 24 cases) for the first time after induction chemotherapy, 30 patients (56.6 %) obtained complete remission (CR), 23 cases (43.4 %) did not remission (NR). 12 cases of patients with Myelodysplastic syndrome (MDS) turn AEL CR rate was 33.3 %, significantly lower than the primary AEL group ($P < 0.05$). 29 cases of male patients, 15 cases (51.7 %) for CR. 24 cases of female patients, 15 cases (62.0 %) for CR. (2) Allogeneic hematopoietic stem cell transplantation: 23 patients pre-treatment solutions using modified BUCY, All patients were successfully implanted, the median granulocyte series reconstruction time was 12 days (1, 1–14), the median platelet reconstruction time was 12 days (8–15). Until at the end of follow-up, 4 cases of the transplant patients died, all the rest of the 19 cases survived. 2 cases of recurrence after transplantation, the recurrence time were 3 and 8 months after transplantation, respectively, all died within 1 year after transplantation. Patients with recurrence after transplantation in 2 cases were complicated abnormal karyotype, two courses of induced chemotherapy did not achieve CR, and they belong to the refractory cases.

Survival Analysis

53 patients with long-term follow-up, the median survival time was 33 months. The OS rate of 13–39 years was highest, the OS rate of 40–49 years was second, more than 50 years old patients with worst OS rate (Fig. 1). Women were superior to men in OS rate ($P = 0.047$) (Fig. 2). The OS rate of the patients with normal karyotype was superior to the abnormal group, complex OS rate of the complex abnormal group was worst ($P < 0.01$) (Fig. 3). For the patients with remission after first induction therapy, the OS rate was better than not remissions ($P < 0.05$) (Fig. 4), OS rate of transplantation group was obviously higher than that of the chemotherapy group ($P < 0.05$) (Fig. 5).

Fig. 1 Comparison of overall survival between different age groups

Fig. 2 Comparisons of overall survival between different genders

Fig. 3 Comparisons of overall survival between different karyotypes

Discussion

AEL as a subtype of AML has its unique biological features. [6–8]. Literatures reported that more than 46 % of the patients with AEL appeared tumor-like syndrome

Fig. 4 Comparisons of overall survival between remission and non-remission

Fig. 5 Comparisons of overall survival between transplantation group and chemotherapy group

(fever, night sweats, weight loss) [9, 10]. In this study, AEL patients characterized by symptoms of red blood cells reduce, granulocytes reduce and megakaryocyte reduce, and often with fever, skin and mucosal bleeding. Thus the diagnosis relies mainly on bone marrow cytology and immunophenotyping [11].

So far, there was no finding about AEL specificity abnormal karyotype. In this study, the abnormal chromosome karyotype rate was 37.8 %, less than 62.3 % (17/27) that reported by liujin et al. [12].

In this study, 56 cases of abnormal karyotype, +8 was the most common followed by 5q-. 87 cases of male patients, an abnormal karyotype detection rate was 40.2 % (36/87), including 19 cases complicated abnormal karyotype. 61 cases of female patients, an abnormal karyotype detection rate was 36.1 % (22/61), including 8 cases complicated abnormal karyotype. According to the AML prognostic stratification, normal karyotypes and +8, 7q were medium prognostic markers, 5q-5, -5, -7, 3q and complex karyotype were adverse prognostic markers. But

30 cases of +8 abnormal, pure +8 abnormal only 11 cases, the rest of the 19 cases were merged with other chromosomal abnormalities. Good prognosis karyotype in AEL was rare, which can be seen from the results that AEL prognosis was worse than other types of AML.

Current research suggests NPM1 gene mutations in AML were the highest incidence of a molecular genetic change type [13]. In 2005, Falini et al. [14] studied the relationship between 12th exon mutations of NPM1 gene with AML. Results showed that the mutation detection rate was 35.2 % in adult AML, and the detection rate was 61.7 % in normal karyotype AML. The results showed that the NPM mutation rate in the normal karyotype AEL was only 15.4 % among patients, the AML mutation rate was lower than the foreign literature report. At present, the reports of CEBPA concentrated in normal karyotype AML, few literatures about AEL CEBPA mutation, our data showed that 44 % (11/25) patients with AEL CEBPA mutation. Mutations were TAD1 area and b-ZIP area double mutation. Previous studies reported that normal karyotype AML merger CEBPA mutation was a sign of good prognosis [15], but the mutation cases in this paper were less, therefore, there is need to expand the sample to continue test and verify.

This study AEL treated first induced the CR rate was 56.6 %, close to 54 % of reported by Colita et al. [16]. Survival analysis showed that the greater age, the worse overall survival. The result was consistent with previous reports. The OS rate of AEL female patients was higher than male, this may be related to abnormal karyotype and complex abnormal karyotype of female patients was lower than men, and the first induction chemotherapy remission rate was higher than male patients. Allogeneic hematopoietic stem cell transplantation patients with AEL, the OS was superior to conventional chemotherapy ($P < 0.01$). Therefore, we recommend that AEL patients with remission after chemotherapy should be for allogeneic hematopoietic stem cell transplantation treatment as early as possible.

In conclusion, AEL has unique biological features; allogeneic hematopoietic stem cell transplantation can significantly improve the prognosis of patients.

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Compliance with Ethical Standards

Conflict interest The authors declare no conflict of financial interest.

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