Leukemia Research 70 (2018) 13–19

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Shifting of erythroleukemia to myelodysplastic syndrome according to the revised WHO classification: Biologic and cytogenetic features of shifted erythroleukemia

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ARTICLE INFO

Keywords:
Acute erythroleukemia
2008 WHO
2016 WHO
Cytogenetics

ABSTRACT

The 2016 revision of the World Health Organization (WHO) classification of tumours of haematopoietic and lymphoid tissues was published. According to 2016 WHO criteria, diagnostic criteria of acute erythroid leukemia was revised. We reassessed 34 de novo acute erythroid leukemia (AEL) diagnosed by 2008 WHO criteria, according to 2016 WHO criteria. A total of 623 patients (excluding M3) with acute myeloid leukemia including 34 patients with AEL were enrolled. Among 34 patients diagnosed with AEL, diagnosis was shifted to MDS-EB in 28 patients (28/34, 82.3%) and MDS-U in 2 patients (2/34, 5.9%), while remained as AEL in 4 patients (4/34, 11.8%) according to 2016 WHO criteria. Interphase FISH for cytogenetic changes of MDS (-5/del(5q), −7/del (7q), del(20q), +8) revealed cytogenetic aberrations in 50.0% (17/34) of AEL 2008 group. AEL 2008 group showed higher frequency of complex cytogenetic abnormalities and higher MDS related cytogenetic abnormalities than AML excluding AEL group. Transformation to another AML subtype was noted in 10% in AEL shifted to MDS. Majority (88.2%) of AEL by 2008 WHO criteria was reclassified to MDS by 2016 WHO criteria. Clinical characteristics of shifted AEL were similar to those of MDS rather than de novo AML.

1. Introduction

Acute erythroid leukemia (AEL) is a rare leukemia characterized by the predominance of dysplastic erythroid population and coexisting increase of myeloblasts. According to the diagnostic criteria of 2008 World Health Organization (WHO) classification, AEL is diagnosed when erythroid cells are ≥50% of bone marrow (BM) nucleated cells and when the blasts are ≥20% of the non-erythroid cells but less than 20% of the total BM nucleated cells [1]. Erythroblasts are not included in blast count and have deeply basophilic cytoplasm with occasional vacuoles. Erythroid cells are characterized by dysplastic morphology with occasional granular PAS positivity. Dysplasia widely spans megaloblastic changes, nuclear budding, multinuicleation, karyorrhexis, internuclear bridging, ring sideroblasts, coarse stippling, and cytoplasmic vacuolization. Patients often present with cytopenia and BM failure symptoms [2]. However, these findings are not unique to AEL, also being observed in myelodysplastic syndromes (MDS), acute myeloid leukemia (AML) with myelodysplasia-related changes (MRC), and even reactive erythroid hyperplasia. Differential diagnosis among myelodysplastic syndrome-excess blasts (MDS-EB), AML with MRC, AEL has been challenging and the parameters used for differential diagnosis were myeloblast% among all the nucleated cells in BM, history of prior therapy, and the presence of recurring WHO genetic abnormalities. Specific morphological, cytogenetic or molecular characteristics of AEL have not been elucidated, but AEL frequently harbors complex chromosomal abnormalities involving the chromosomes 5 and 7. Another study suggested that AEL may be the same disease entity as MDS transformation based on cytologic, karyotypic and clinical characteristics [3] and a recent study implies that AEL is in the continuum of MDS and AML with erythroid hyperplasia [4]. The big difference between the 2001 and 2008 WHO classification of AML is the introduction of the AML with MRC in the AML category.

The 2016 WHO classification presented revised version and redefined AEL. The biggest difference is that the counting of blast percentage is based on total BM cells in AEL alike all the other AML subtypes (revised erythroleukemia criteria). Similar to 2008 WHO,

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https://doi.org/10.1016/j.leukres.2018.04.015
Received 19 October 2017; Received in revised form 26 April 2018; Accepted 27 April 2018
Available online 30 April 2018
0145-2126/

erythroblasts are not included in blast%. Since the criteria of AEL 2008 version adopted the blast > 20% among noneryhtroid cells in BM, minor differences in myeloblast% can rediagnose the patients from myelodysplastic syndrome to acute myeloid leukemia. Furthermore, during the monitoring of cases with MDS showing erythroid hyperplasia, a slight increase in myeloblast count might lead switch to the diagnosis from MDS to AEL, which means disease progression.

We investigated what proportion of AEL diagnosed by 2008 WHO criteria is reclassified as MDS-EB by 2016 WHO criteria and whether clinicopathologic features of shifted AEL is similar to MDS. According to the revised criteria of 2016 WHO criteria, we reclassified 34 de novo AEL patients who were diagnosed with AEL by 2008 WHO criteria and compared the clinicopathological and cytogenetic characteristics between AEL shifted to MDS (shifted AEL) and sustained AEL. Also, we compared the clinicopathologic features of sustained AEL with the other AML subtypes (AML excluding AEL).

2. Materials and methods

2.1. Patients

A total of 623 Korean adult patients (≥15 years) diagnosed with de novo AML (excluding M3) at Seoul National University Hospital between January 2000 and December 2015 were enrolled. Clinical information including diagnosis, laboratory results, and treatment was retrospectively gathered through hospital electronic medical record. All cases were diagnosed with peripheral blood smears, BM aspirates, trephine biopsies and/or flow cytometry, and cytogenetic studies. Diagnosis of AML subtypes was based primarily on the 2008 WHO criteria [1] and were reclassified according to the revised 2016 WHO criteria [5].

2.2. Bone marrow histological examination

Hematopathologists reviewed Wright-stained BM smears and hematoxylin and eosin (H&E)-stained sections of BM trephine biopsies. The percentages of blasts, the presence of ring sideroblasts and the morphologic dysplasia in each hematopoietic lineage were determined in BM smears. For the BM sections, blast infiltration and megakaryocyte numbers were determined. In all sections, immunohistochemical staining was performed for CD34, CD117 and CD61 (all from Dako, Glostrup, Denmark).

2.3. G-banding analysis

Cytogenetic analysis was performed in 607 patients with G-banding and/or interphase FISH for four recurrent genetic abnormalities of AML and MDS. In 607 patients, G-banding was carried out after direct harvest or short-term culture of BM cells [6]. Clonal abnormalities were defined and described according to the International System for Human Cytogenetic Nomenclature [7].

2.4. FISH

Interphase FISH for four recurrent chromosomal abnormalities in AML, *RUNX1-RUNX1T1* rearrangement, *PML-RARA* rearrangement, *MLL* rearrangement, inv(16), were performed in 323 AML excluding AEL cases and 15 AEL cases. The probes used were Vysis LSI AML1/ETO, PML/RARA, MLL, CBFB (Abbott laboratories, Illinois, USA).

Interphase FISH for cytogenetic changes of MDS (-5/del(5q), −7/del(7q), del(20q), +8) were performed in 18 AEL cases. To evaluate numerical changes in AEL patients, 11 other FISH tests were done. Primarily, chromosomal enumeration probes (CEP) were used and in case where there was no CEP available, probes assessing chromosomal rearrangements were surrogately used. The probes used were Vysis LSI 1p36/1q25, LSI ALK Dual Color, LSI BCL6 Dual Color, LSI CHIC2, LSI EGR1(5q31)/D5S23(5p15.2), LSI MYB/RREB1/CEP6, LSI D7S522(7q31)/CEP 7, LSI IGH/MYC, CEP 8, LSI BCR/ABL DC, DF, CEP 12, LSI D13S25(13q14.3), LSI IGH/BCL2, LSI 19q13/19p13, LSI D20S108(20q12), and CEP X/Y (Abbott laboratories, Illinois, USA). Increased copy number in the absence of fusion signal was considered hyperdiploid of the corresponding chromosome. FISH procedure was performed according to the manufacturer's instruction and at least 200 interphase cells were counted for signal patterns for chromosomal rearrangements and numerical chromosomal abnormalities.

2.5. PCR assays for FMS-like tyrosine kinase 3 internal tandem duplication (FLT3-ITD) mutation

Genomic DNA was extracted from bone marrow samples using the PureGene DNA Isolation Kit (Gentra Systems, Minnesota, USA). Amplified products were sequenced using an ABI 3730 analyzer (Applied Biosystems, California, USA), using the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems). FLT3exons 14 through 15 were amplified using forward primer 5′-CCCTTCCCTTTCA TCCAAGA-3′ and reverse primer 5′-AACTGTGCCTCCCATTTTTG-3′. The sequences were analyzed using SeqScape version 2.5 (Applied Biosystems) and Sequencher version 5.0 (Gene Codes Corporation, Michigan, USA).

2.6. Statistical analysis

Chi-square test and Mann-Whitney test was used for comparisons between groups. Survival was estimated by Kaplan-Meier method from the date of pathological diagnosis until death or the last follow up date compared with the log rank test. Multivariate Cox regression analysis was done to evaluate the risk factors for survival. SPSS 23 (SPSS Inc., Chicago, Illinois, USA) was used for statistical evaluation. All *P*-values are two sided and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Clinical parameters

The distribution and the clinical characteristics of AML patients according to 2016 WHO classification are shown in Table 1. According to the FAB classification, 3.5% of the cases were classified in M0, 11.2% of the cases were classified in M1, 33.5% in M2, 21.3% in M4, 7.9% in M5, 5.5% in M6, and 1.0% in M7. 16.1% of the cases were not classified in FAB subtypes. Comparing the clinical parameters between AML excluding AEL and AEL 2008, AEL 2008 patients tend to have higher age at diagnosis with a median of 61.5 (17.0-84.0) years versus 55.0 (15.0-89.0) years for AML excluding AEL patients, though statistically not significant (P = .076). The WBC count was higher in the AML excluding AEL group ($P < 0.001$) with median of 33,677 (1700.0-434800.0) $x10^3$/mL and 4043 (590.0-32,900.0) $x10^3$/mL for AEL 2008. The hemoglobin level and platelet count were not significantly different between the two groups ($P = 0.350$, $P = 0.923$, respectively).

3.2. Results of bone marrow histologic examination

The results of bone marrow histologic examination of all AML cases are summarized in Table 1. Comparing the data between AML excluding AEL and AEL 2008, Erythorid% in bone marrow was significantly higher in AEL2008 group ($P = 0.000$) with median 64.0% (47.7-87.4) and 5.3% (0.0-66.4) for AML excluding AEL group. In AML excluding AEL group, M/E ratio, blast% of total cells, and blast% of non-erythroid cells are significantly higher than AEL 2008 group [3.0 (0.0-479.0) vs. 0.3 (0.0-1.0), $P = 0.000$, 62.8% (2.4-99.0) vs. 12.5% (0.3-22.1), $P = 0.000$, and 69.3% (0.4-100.0) vs. 37.0% (1.2-82.8), $P = 0.000$, respectively). In sustained AEL group, Blast% of total cells and Blast% of non-erythroid cells are significantly higher than shifted