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Case report

High incidence of disseminated intravascular coagulation and acute cerebral infarction in acute myeloid leukemia with cup-like nuclei

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In this study, we examined a cohort of Japanese patients with acute myeloid leukemia (AML) with cup-like nuclei. In particular, we attempted to provide a detailed definition of the clinical features of AML with cup-like nuclei. The clinical records of patients diagnosed with de novo AML were collected retrospectively. We showed that approximately 23% of all patients with AML diagnosed during the study period had AML with cup-like nuclei. All three cup-like AML cases had *FLT3*-ITD mutations. In addition, we reported a high incidence of disseminated intravascular coagulation and acute cerebral infarction in patients with AML with cup-like nuclei. Our results show that AML with cup-like nuclei may be more common than expected. Due to these unique characteristics, recognition of this morphology is recommended.

Keywords: acute cerebral infarction, acute myeloid leukemia, cup-like nuclei, disseminated intravascular coagulation

INTRODUCTION

According to the World Health Organization (WHO), acute myeloid leukemia (AML) is a complex diagnosis that requires information on the morphologic, immunophenotypic, and molecular/cytogenetic features of leukemic cells. Flow cytometric immunophenotyping is a useful tool for classifying AML. Flow cytometry could help diagnose acute promyelocytic leukemia (APL). It is well known that malignant promyelocytes are characterized by HLA-DR and CD34 negativity.[1] In 2004, Kussick *et al.* described the presence of blasts with cup-like nuclei in AML cases for the first time. They found that AML cases with cup-like nuclei were associated with CD34 and HLA-DR negativity, a normal karyotype, and fms-like tyrosine kinase-internal tandem duplication (*FLT3*-ITD) mutations.[2] In addition, cases of AML with cup-like nuclei had been reported to be associated with the female sex, higher complete remission (CR) rates, French-American-British (FAB) AML-M1, high total leukocyte count, a high bone marrow blast percentage, the absence of CD7, high D-dimer levels, and myeloperoxidase positivity.[3-5] Due to these unique characteristics, recognition of this morphology is recommended. Herein, we studied a cohort of Japanese patients with AML with cup-like nuclei and attempted to define their characteristics in as much detail as possible.

MATERIALS AND METHODS

This study was a retrospective review of medical records from patients diagnosed with de novo AML at Juntendo University Shizuoka Hospital between April 2017 and September 2017. Cup-like morphology was defined as cup-like nuclear invagination spanning ≥ 25% of the nuclear diameter in > 10% of the blasts, as previously described.[2] Disseminated intravascular coagulation (DIC) was defined according to the criteria of the Japanese Ministry of Health and Welfare.[6] To compare the different patient groups, we applied Fisher's exact test for categorical variables. All p-values ≤ 0.05 were considered significant. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan),[7] which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics. The study protocol was approved by the Ethics Committee of the Juntendo University Shizuoka Hospital.

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CASE REPORT

In total, 13 patients diagnosed with de novo AML during the study period were included. Of these patients, 8 (61.5%) were men. Representative cup-like blasts are shown in Fig. 1A with marked myeloperoxidase positivity (Fig. 1B). May-Grunwald Giemsa bone marrow smears from case 1 demonstrate the cup-like nature of the nuclear invaginations in many of the blasts. The blasts were intermediate-sized with scant cytoplasm. Auer rods were not seen. Prominent nuclear invaginations were identified in > 10% of the blasts in each patient. Overall, 3 patients (23%) were diagnosed with cup-like AML, while 10 patients were diagnosed with other forms of AML and comprised the control group (Table 1). The median age of patients with cup-like AML was 82 (range, 79–83) years. Cup-like AML, when compared to other forms of AML, was associated with a higher leukocyte count (193.8×10^9/L and 4.6×10^9/L, respectively; $p < 0.01$), a higher bone marrow blast percentage (98% and 37.5%, respectively; $p < 0.001$), CD34 negativity (100% and 10%, respectively; $p < 0.001$), and HLA-DR negativity (100% and 0%, respectively; $p < 0.001$). Notably, all differences were statistically significant ($p < 0.05$). All 3 patients with cup-like AML presented with *FLT3*-ITD mutations. They did not have PML-RARA gene fusion and variant mutations of RARA. Moreover, we found that patients with cup-like AML were significantly more likely to develop DIC than patients with other forms of AML (100% vs. 10%, respectively; $p < 0.001$) and acute cerebral infarction (66.7% vs. 0%, respectively; $p < 0.01$). All acute cerebral infarctions were atherothrombotic brain infarctions and occurred after initial treatment. Although female sex and higher CR rates were reportedly associated with cup-like AML in the literature, they were not found to be associated in our cohort (data not shown).

DISCUSSION

We investigated a cohort of Japanese patients with cup-like AML. The prevalence of AML with cup-like nuclei among all patients with AML has been reported to be 1–10%.[3,8] However, the frequency (23%) was higher at our hospital. Although this was a small study conducted in a single institute, our results show that AML with cup-like nuclei may be more common than expected. The prevalence of cup-like AML may be higher in patients in Japan than those in the United States and Europe. AML with cup-like nuclei was not listed in the 2016 revision to the WHO classification of myeloid neoplasms and acute leukemias.[9] Therefore, AML with cup-like nuclei may be underestimated and insufficiently studied. These blasts are typically negative for CD34 and HLA-DR on immunophenotyping and show laboratory evidence of DIC. Therefore, it is possible that cup-like AML may be misdiagnosed as APL,[10] and recognition of this morphology is recommended. In this cohort, cup-like AML was associated with a high leukocyte count, a high bone marrow blast percentage, and CD34 and HLA-DR negativity, similar to previous reports. Sex and response might not have been associated with cup-like AML in this cohort. Notably, we have reported a high incidence of DIC with high fibrinogen degradation products (FDP) in patients with AML with cup-like nuclei. The median FDP value was 81.3 (range, 73.1–101.1) μg/mL (Table 1).

Two of the 3 patients with AML with cup-like nuclei had cerebral infarction. Therefore, we consider that DIC in cup-like AML cases is of a thrombotic phenotype. Tissue factor and annexin II are considered to play a role in the development of DIC in APL.[11] However, the mechanism of DIC in cup-like AML cases is not completely understood. Although cup-like AML blasts are rich in azure granules such as APL, the DIC pattern in cup-like AML is a thrombotic phenotype. Therefore, the mechanism of DIC may differ from that of APL. A normal karyotype and elevated leukocyte count have been reported as risk factors for DIC in non-promyelocytic AML.[12] As cup-like AML cases are associated with a normal karyotype and an elevated leukocyte count, a high incidence of DIC and acute cerebral infarction may be observed. In our study, all 3 patients showed excessive white blood cell counts, which has been reported to be a predictor of cerebral hemorrhage in patients with acute leuke-

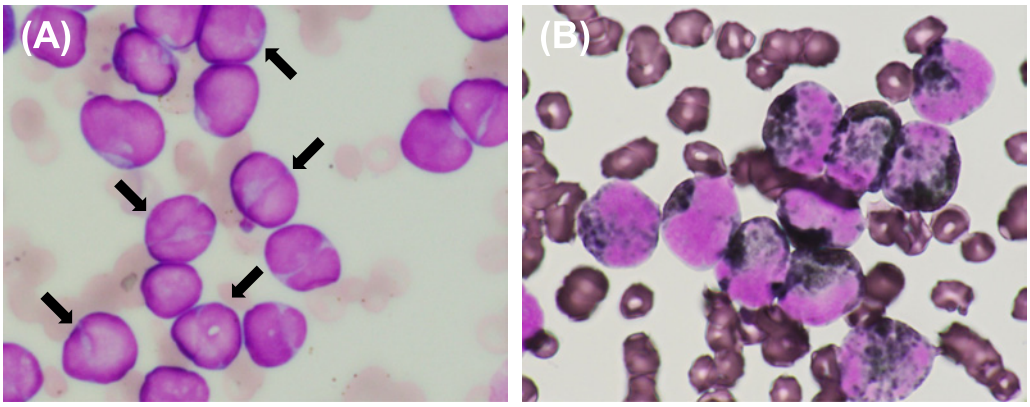

Fig. 1. Blasts showing typical cup-like nuclei
(***A***) Typical blasts with cup-like nuclei (arrows), May-Grunwald Giemsa staining (×1000); (***B***) Blasts showing strong myeloperoxidase positivity, myeloperoxidase staining (×1000)

Table 1. Characteristics of patients with AML

Cup-like AML											
Case No.	Age/Sex	WBC (x 10^9/L)	BM Blasts (%)	FAB subtype	Karyotype	*FLT3*-ITD Mutation	PT-INR	Fibrinogen (mg/dL)	FDP (μg/mL)	Initial treatment	Cerebral infarction
1	79/F	205.9	99	M1	46, XX	+	1.38	183	101.1	cytarabine	-
2	82/F	100.3	98	M1	46, XX	+	1.89	192	73.1	cytarabine	+
3	83/M	193.8	97	M1	46, XY	+	1.22	216	81.3	cytarabine	+

Non-cup-like AML											
Case No.	Age/Sex	WBC (x 10^9/L)	BM Blasts (%)	FAB subtype	Karyotype	*FLT3*-ITD Mutation	PT-INR	Fibrinogen (mg/dL)	FDP (μg/mL)	Initial treatment	Cerebral infarction
1	76/M	1.6	41	M0	46, XY, der(15)t(1;15)(q21;p13)	NA	1.04	336	12.5	cytarabine	-
2	75/M	3.4	30	M6a	47, XY, +1, der(1;7)(q10;p10)	NA	1.08	488	2.9	CA	-
3	77/M	5.8	59	M2	46, XY, ?t(8;21)(q22;q22), del(9)(q?)	NA	1.42	278	2.5	CA	-
4	66/M	1.6	34	M6a	46, XY	NA	1.03	269	1.2	IDR/Ara-C	-
5	62/F	47.7	42	M2	46, XY, t(8;21)(q22;q22)	-	1.2	297	1.8	IDR/Ara-C	-
6	79/F	2.4	23	M2	46, XX	NA	3.21	435	20.5	best supportive care	-
7	69/M	9.4	60	M1	46, XY, add(2)(q21)	-	1.05	309	6.7	IDR/Ara-C	-
8	66/M	1.7	44	M1	46, XY	NA	0.94	223	1.9	IDR/Ara-C	-
9	70/F	45.4	21	M2	46, XX, t(8;21)(q22;q22)	-	1.23	496	10.9	azacitidine	-
10	85/M	140.7	23	M4	47, XY, +1, der(1;7)(q10;p10), inv(3)(q21q26.2)	NA	1.99	86	5.0	best supportive care	-

Abbreviations: F, female; M, male; WBC, white blood cell; BM, bone marrow; FAB, French-American-British; *FLT3*-ITD, fms-like tyrosine kinase-internal tandem duplication; PT-INR, prothrombin time international normalized ratio; FDP, fibrinogen degradation products; NA, not available; CA, cytarabine and aclarubicin; IDR/Ara-C, idarubicin and cytarabine.

mia.[13] The correlation between cerebral infarction and hyperleukocytosis in non-APL AML patients is unclear; blasts with cup-like nuclei may be an important risk factor for cerebral infarction. A limitation of this study was that we did not measure D-dimer, plasmin-alpha2-plasmin inhibitor complex, and thrombin-antithrombin complex.

Patients with cup-like AML have been reported to be treated with therapy consisting of anthracycline and cytarabine.[10] However, appropriate management for cup-like AML and DIC was not established. Anticoagulant therapy in addition to treatment for underlying disease is recommended for DIC management.[14] Therefore, prompt anticoagulant therapy in addition to adequate chemotherapy for AML was considered a valid option.

Although cup-like AML was reportedly associated with higher CR rates, the high degree of *FLT3* mutations was regarded as a negative prognostic marker. Moreover, some studies have shown that blasts with cup-like nuclei did not affect the survival parameters.[3-5,10] In our study, only 1 cup-like AML case achieved CR after initial treatment, and, therefore, prognosis in such cases remains controversial. While we did not examine nucleophosmin (*NPM1*) mutations, cup-like AML cases have been reported to be associated with a high incidence of *NPM1* mutations in addition to *FLT3* mutations.[4] To elucidate the prognosis of cup-like AML cases, further studies are warranted. AML with *NPM1* mutation was listed in the 5th edition of the WHO Classification of Haematolymphoid Tumours and the new International Consensus Classification (ICC) of myeloid neoplasm and acute leukemia.[15] In the updated classifications, the blast cutoff was changed for the diagnosis of acute leukemia. AML with *NPM1* mutation is considered to be acute leukemia without regard to blast cell count in the WHO classification. AML with mutated *NPM1* requires at least 10% blasts for diagnosis in the ICC. Although it is still unclear how NPM1 mutation contributes to the leukemogenesis of cup-like AML, this type of AML has now been brought into the limelight, along with updates to its diagnostic criteria.

In conclusion, we report that AML with cup-like nuclei may be more common than expected, and there was found to be a high incidence of DIC and acute cerebral infarction in patients with AML with cup-like nuclei in a Japanese cohort. Due to these unique characteristics, recognition of this morphology is recommended.

AUTHOR CONTRIBUTIONS

Naoki Watanabe: Designing the research, analyzing the results, writing the paper. Hideaki Kitahara: Analyzing the results. Tadahiro Honda: Analyzing the results. Hisayo Iwasaki: Analyzing the results. Noriaki Iwao: Analyzing the data. Norio Komatsu: Directing the research. Michiak Koike: Directing the research.

CONFLICT OF INTEREST

The authors declare no financial support or relationships that may pose a conflict of interest relevant to the submitted manuscript.

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