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CASE REPORT

ERYTHROLEUKEMIA*(Eritroleukemia)***Ailinda Theodora Tedja, Riadi Wirawan****ABSTRAK**

Leukemia eritroid akut dibagi menjadi dua (2) subtipen berdasarkan penggolongan World Health Organization (WHO) 2008, yaitu eritroleukemia dan pure erythroid leukemia. Penggolongan WHO 2008 menganjurkan diagnosis leukemia eritroid akut cukup berdasarkan hasil menilai sumsum tulang, berdasarkan jumlah eritroblas dan sel blas, keberadaan diseritropoiesis, serta disgranulopoiesis. Eritroleukemia merupakan bentuk leukemia mieloid akut yang jarang terjadi, yaitu <5% kasus leukemia mieloid akut. Eritroleukemia terutama terjadi di orang dewasa dan lebih sering terjadi di laki-laki. Satu kasus dilaporkan perempuan berusia 42 tahun dengan hasil memerlukan pancytopenia di laboratorium, kemudian dinilai sumsum tulangnya dan didapatkan eritroblas 67% All Nucleated Cells (ANC), disertai diseritropoiesis yang jelas dan blas 25% Non-Erythroid Cells (NEC) dengan disgranulopoiesis 35%. Hasil ini menetapkan diagnosis eritroleukemia (AML-M6) berdasarkan penggolongan WHO 2008. Hasil memerlukan imunofenotip flow cytometry didapatkan komponen eritroid dan mieloid yang mendukung diagnosis eritroleukemia. Pemeriksaan imunofenotip dengan flow cytometry tersebut sebenarnya tidak diperlukan untuk menetapkan diagnosis eritroleukemia. Pemeriksaan sitogenetika disarankan untuk menentukan peramalan perjalanan penyakit.

Kata kunci: AML-M6, eritroleukemia, leukemia eritroid akut, pure erythroid leukemia, imunofenotip flow cytometry

ABSTRACT

According to the 2008 World Health Organization (WHO) classification, acute erythroid leukemia is divided into 2 subtypes i.e. erythroleukemia and pure erythroid leukemia. The 2008 WHO classification recommended that the diagnosis of acute erythroid leukemia can be established only by bone marrow evaluation, based on erythroblast count, blast count, dyserythropoiesis and dysgranulopoiesis. Erythroleukemia is an uncommon type of Acute Myeloid Leukemia (AML), representing less than 5% cases of AML. Erythroleukemia mainly affects the adult population with male predominance. This is a case of a 42-year-old female with pancytopenia and bone marrow evaluation showing 67% erythroblasts of All Nucleated Cells (ANC) with prominent dyserythropoiesis and 25% blasts of Non-Erythroid Cells (NEC) with 35% dysgranulopoiesis. This result established the diagnosis of erythroleukemia (AML-M6) based on the 2008 WHO classification. Flow cytometric immunophenotypic analysis showed erythroid and myeloid components which supported the diagnosis of erythroleukemia. Actually, flow cytometric immunophenotypic analysis is not required to establish the diagnosis of erythroleukemia. However, Cytogenetic analysis is recommended to determine prognosis.

Key words: AML-M6, erythroleukemia, acute erythroid leukemia, pure erythroid leukemia, flow cytometric immunophenotypic

INTRODUCTION

In 1923, Giovanni Di Guglielmo¹ found two (2) forms of acute erythroid leukemia, (ie erythroleukemia and pure erythroid leukemia). Erythroleukemia is also called as erythroid/myeloid or Di Guglielmo syndrome, whereas pure erythroid leukemia is also called Di Guglielmo disease.^{1,2} Erythroleukemia is a form of acute myeloid leukemia rarely found, only as much

as <5%. Erythroleukemia mainly occurs in adults aged > 50 years and more frequently found in males. Meanwhile, pure erythroid leukemia can occur at any age, including infants and children.^{1,3}

French-American-British (FAB) in 1976 classified acute erythroid leukemia as Acute Myeloid Leukemia (AML)-M6 with erythroblasts of ≥30% All Nucleated Cells (ANC) with a dyserythropoiesis of ≥10%.

FAB in 1985 changed the criteria of AML-M6 into erythroblasts of $\geq 50\%$ ANC accompanied with clear dyserythropoiesis and myoblasts of $\geq 30\%$ Non-Erythroid Cells (NEC). Pure erythroid leukemia did not meet the criteria of AML-M6, thus classified into Refractory Anemia With Excess Blasts in transformation (RAEB-t).^{1,2,4}

Kowal-Vern *et al.*⁵ in 2000 proposed three (3) subtypes of AML-M6, as shown in Table 1.

Classification recommended by the WHO in 2001 aimed to classify myeloid malignancies based on its morphology, namely immunophenotype, cytogenetic and molecular. Meanwhile, myeloid malignancies for erythroid series, called acute erythroid leukemia are classified into two (2) subtypes. First, erythroleukemia is characterized by erythroblasts of $\geq 50\%$ ANC accompanied by clear dyserythropoiesis and myoblasts of $\geq 20\%$ NEC. Second, pure erythroid leukemia is characterized by erythroblasts of $\geq 80\%$ ANC accompanied by clear dyserythropoiesis.^{1,2,4}

Next, classification proposed by the WHO in 2008 still divided acute erythroid leukemia into two (2) subtypes and aimed to establish a criteria for

making the diagnosis of acute erythroid leukemia, ie get rid of comparative diagnosis, such as AML with Myelodysplasia-Related Changes (AML-MRC), therapy-related AML (t-AML) and reactive erythroid hyperplasia due to erythropoietin treatment.^{1,2,4} The classifications proposed by FAB and WHO for acute erythroid leukemia briefly can be seen in Table 2.

Clinical symptoms of acute erythroid leukemia are not typical, ie pale, weakness, fever, and bleeding. On physical examination, hepatosplenomegaly can be found.^{1,2} Normochromic normocytic anemia and thrombocytopenia can also be found almost in all cases. Erythrocyte abnormalities that are not typical, such as anisopoikilocytosis, anisochromia and basophilic stippling can be found in the peripheral blood. Besides that, neutrophil count varies from normal to low, and Pelger pseudo-Huet abnormality can be found. Blasts in peripheral blood also become various and sometimes can not be found.^{1,2}

Cell density of bone marrow in erythroleukemia is usually hypercellular. Aspiration and living tissue sampling of bone marrow show an increased number of immature erythroblasts. Dyserythropoiesis include

Table 1. Subtypes of AML-M6 by Kowal-Vern *et al.*⁵

Subtypes	Bone Marrow		
	% erythroblast of ANC	% Proerythroblast of Erythroid Components	% Myeloblasts of NEC
Erythroleukemia (M6a)	$\geq 50\%$	<30%	$\geq 30\%$
Pure erythroid leukemia (M6b)	$\geq 50\%$	$\geq 30\%$	<30%
Myeloblast- and proerythroblast-rich mixed variant (M6c)	$\geq 50\%$	$\geq 30\%$	$\geq 30\%$

Table 2. Classifications of FAB and WHO for acute erythroid leukemia^{1,2,4}

Classification	Year	Subtypes	Bone Marrow			Note
			% Erythroblast of ANC	% Blasts of NEC	Dyserythropoiesis	
FAB	1976	AML-M6	$\geq 30\%$	–	$\geq 10\%$	–
	1985	AML-M6	$\geq 50\%$	$\geq 30\%$	Clear/Obvious	
WHO	2001	Erythroleukemia (Erythroid / Myeloid)	$\geq 50\%$	$\geq 20\%$	Clear/Obvious	Dysplasia <50% myeloid cells and megakaryocytes
		Pure Erythroid Leukemia	$\geq 80\%$	–	Clear/Obvious	
	2008	Erythroleukemia (Erythroid / Myeloid)	$\geq 50\%$	$\geq 20\%$	Clear/Obvious	Out of AML-MRC, t-AML, erythropoietin therapy
		Pure Erythroid Leukemia	$\geq 80\%$	–	Clear/Obvious	

Abbreviations: AML, acute myeloid leukemia; AML-MRC, AML with myelodysplasia-related changes; t-AML, therapy-related AML

megaloblastoid shape, nuclear budding, nuclear bridging, irregular cell nucleus, a cell nucleus of ≥ 2 , karyorrhexis and cytoplasmic vacuolization. Auer rods may be seen in myeloblasts. Granulocytic line dysplasia and regular megakaryocytes usually can be found.¹⁻³

Erythroblasts express transferrin receptor-1 (CD71), thrombospondin receptor (CD36) and glycophorin A/GlyA (CD235a). CD36 is usually positive in immature erythroblasts. CD36 is not specific to erythroblasts and can be expressed by monocytes and megakaryocytes. Erythroblasts also express CD235a, especially in more mature erythroblasts. The population of myeloblasts in erythroleukemia then expresses myeloid markers, such as MPO, CD117, CD13, and CD33, with or without the expressions of CD34 and HLA-DR.^{1-3,6,7}

In addition, chromosomal abnormalities in acute erythroid leukemia can also be used to determine prediction for the disease progression. However, there are no specific chromosomal abnormalities in acute erythroid leukemia. Chromosomal abnormalities most often found are monosomy 5, del (5q), monosomy 7, del (7q) and trisomy 8. The complex karyotype

with more than or as many as three chromosomal structural abnormalities is also often found. As a result, patients with -5 / del (5q), -7 / del (7q), inv (3q) and complex karyotypes are predicted to have a bad progression of the disease, namely the less the response to chemotherapy, the shorter the lifespan of the patients.¹⁻⁴

Predicted progression of pure erythroid leukemia, furthermore, is worse than erythroleukemia. Estimated lifespan of erythroleukemia patients is about 25 months, while in pure erythroid leukemia about 3 months. Pure erythroid leukemia, therefore, is associated with bad cytogenetic abnormalities.^{1,3}

CASE

42-year-old female was examined for her bone marrow without any clinical information. The results of the laboratory examination showed hemoglobin levels at 2.0 g/dL, leukocyte count of 1650/uL and platelet count of 25,000/uL.

Overview of bone marrow examination

Preparations: Wright

Particle: No

Cell density: hypercellular

Fat cells: Fewer

Thrombopoiesis: megakaryocytes, hard to find, and less formation of platelets

	Total (%)	Reference values
Neutrophils eutrophic granulopoiesis	30	40.1–65.5
Blasts	7.5	0.3–1.3
Promyelocyte	1.0	0.9–3.7
Myelocyte	2.0	9.9–19.5
Metamyelocyte	7.0	11.3–23.4
Trunk	5.0	6.2–15.5
Segment	4.5	3.6–11.9
Basophils	0	0–0.4
Eosinophils	3.0	0.9–4.7
Monocytes	0	0.5–2.2
Erythropoiesis	67	22.3–44.9
Proerythroblasts	15	0.1–1.7
Normoblasts	27	1.2–3.9
Rubricyte	19	8.0–18.3
Metarubricyte	6	11.4–29.6
Lymphoid system	3	5.3–21.7
Lymphocytes	3	4.5–19.1
Plasmocytes	0	0.3–2.2
The ratio of M: E	1:2	1.0–4.1

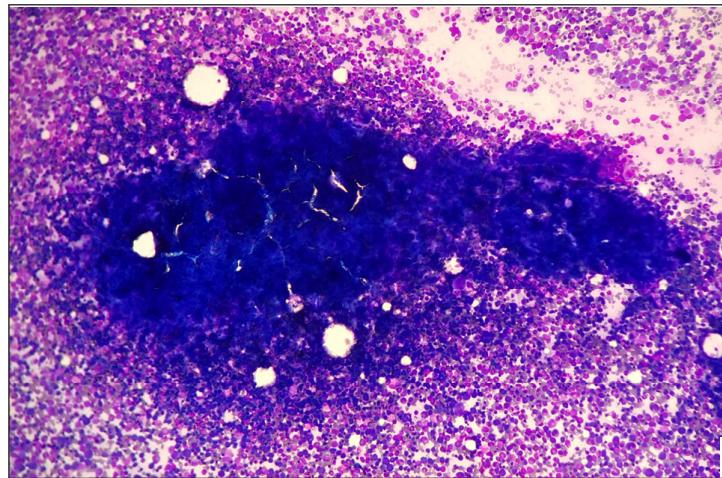


Figure 1. Hypercellular bone marrow.

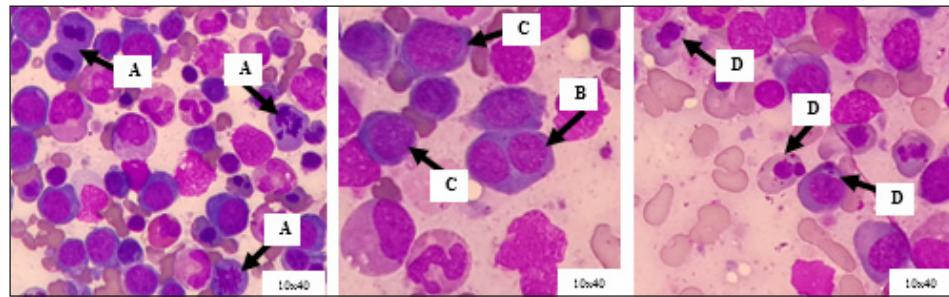


Figure 2. Dyserythropoiesis

- (A) Mitosis
- (B) Binucleated
- (C) Maturation of megaloblastoid
- (D) Karyorrhexis core

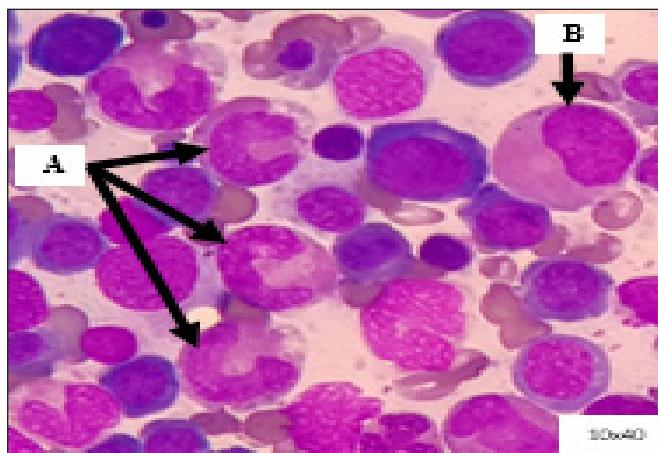


Figure 3. Ekstralinguisis

- (A) Giant stab
- (B) Giant metamyelocyte

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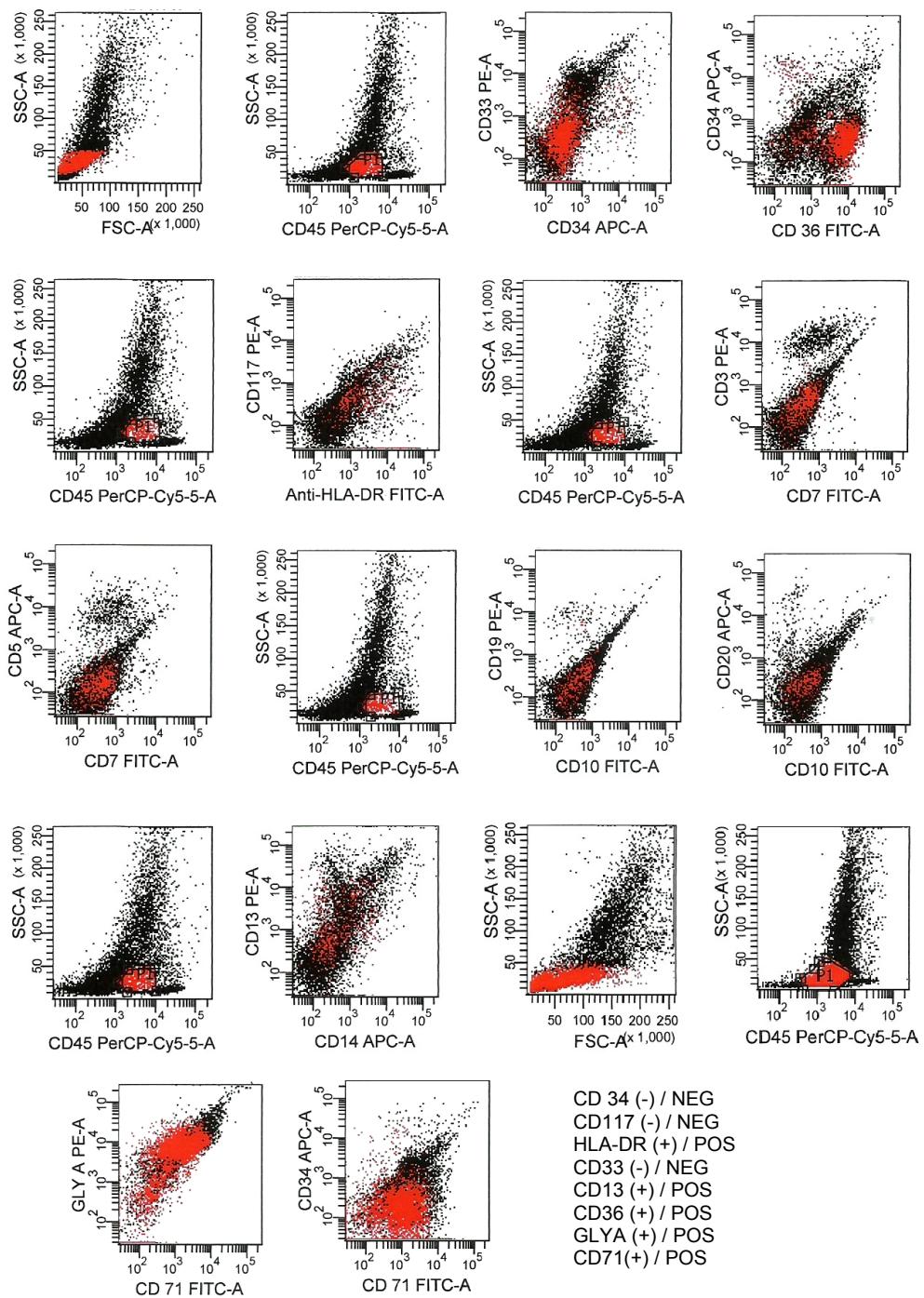


Figure 4. The results of immunophenotyping test by flow cytometry.

In the assessment of her bone marrow, it was known that her bone marrow was hypercellular. Activities of thrombopoiesis were also depressed. Erythropoiesis was hyperactive with erythroblasts of 67% ANC, accompanied with certain dyserythropoiesis, such as megaloblastoid maturation, karyorrhexis core and binucleated. The number of blast cells found was 7.5% ANC or 25% NEC. Dysgranulopoiesis was also found in the form of giant stab and giant metamyelocyte, about 35% of the granulocyte cells. The ratio of M: E found was 1: 2. Therefore, the results of assessing this bone marrow met the criteria of erythroleukemia proposed by WHO in 2008.

The results of immunophenotyping test by flow cytometry, furthermore, found abnormal cell populations with low FSC and SSC, mid and high expressions of CD45, erythroid markers (CD36, GlyA and CD71+), and myeloid markers (HLA-DR, and CD13+). Components of erythroid and myeloid found in the immunophenotyping test by flow cytometry also supported the diagnosis of erythroleukemia. The results of immunophenotyping test by flow cytometry can be seen in Figure 4.

DISCUSSION

The classification acute erythroid leukemia diagnosis as recommended by WHO in 2008 was based on bone marrow assessment, the number of erythroblasts of the ANC, the number of blast cells of NEC, the existence of a clear and obvious dyserythropoiesis and dysgranulopoiesis.^{1,2,4}

This case was about a 42-year-old female suffering from erythroleukemia. This case actually rarely occurs, only in <5% of AML cases. She then was asked to have pancytopenia examination in laboratory tests for checking her bone marrow and undergo immunophenotyping test by flow cytometry. In the assessment of her bone marrow, the results showed erythroblasts of 67% ANC, accompanied by clear dyserythropoiesis and blasts of 25% NEC with 35% dysgranulopoiesis. It means that these results met the criteria of erythroleukemia proposed by WHO in 2008.

In addition, the results of immunophenotyping test by flow cytometry showed abnormal cell populations with erythroid markers (CD36, GlyA, and CD71+) and myeloid markers (HLA-DR and CD13+), supporting the diagnosis of erythroleukemia. Nevertheless, cytogenetic examination is still recommended to determine the further prediction of the disease progression.

CONCLUSION

This article presented a case of a 42-year-old female who suffered from pancytopenia, similar with erythroleukemia subtype of acute erythroid leukemia based on the criteria recommended by WHO in 2008. The results of assessing her bone marrow showed erythroblasts of 67% ANC, accompanied by clear dyserythropoiesis and blasts of 25% NEC with 35% dysgranulopoiesis. Actually, it is not necessary to conduct immunophenotyping test by flow cytometry to establish the diagnosis of acute erythroid leukemia.

REFERENCES

1. Mihova D, Zhang L. Acute erythroid leukemia: a review. *N A J Med Sci.* 2012; 5(2): 110-8.
2. Zuo Z, Polski JM, Kasyan A, Medeiros J. Acute erythroid leukemia. *Arch Pathol Lab Med.* 2010; 134(9): 1261-70.
3. Arber DA, Brunning RD, Orazi A, Porwit A, Peterson L, Thiele J, et al. Acute myeloid leukemia, not otherwise specified. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. *WHO classification of tumours of haematopoietic and lymphoid tissues.* 4th Ed., Lyon, International Agency for Research on Cancer; 2008; 134-6.
4. Hasserjian RP, Zuo Z, Garcia C, Tang G, Kasyan A, Luthra R, et al. Acute erythroid leukemia: a reassessment using criteria refined in the 2008 WHO classification. *Blood.* 2010; 115: 1985-92.
5. Kowal-Vern A, Mazzella FM, Cotelingam JD, Shrit MA, Rector JT, Schumacher HR. Diagnosis and characterization of acute erythroleukemia subsets by determining the percentages of myeloblasts and proerythroblasts in 69 cases. *Am J Hematol.* 2000; 65(1): 5-13.
6. Leach M, Drummond M, Doig A. Practical flow cytometry in haematology diagnosis. 1st Ed., Oxford, John Wiley & Sons, 2013; 48-74.
7. Craig FE, Foon KA. Flow cytometric immunophenotyping for hematologic neoplasms. *Blood.* 2008; 111: 3941-67.