Morphological characteristics of leukemia cells in acute myeloblastic leukemia with t(8;21)(q22;q22): possible predictability of t(8;21)

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Abstrak

Sistim laboratorium untuk analisis kromosom ataupun deteksi fusi gen secara umum masih kurang berkembang di Indonesia. Karena itu, metoda analisis morfologis sumsum tulang perlu dikembangkan dan diaplikasikan untuk diagnostik semaksimal mungkin. Untuk itu, kami melakukan analisis morfologis dari sediaan sumsum tulang delapan (8) kasus leukemia mieloblastik akut (LMA) dengan translokasi (8;21)(q22;q22) untuk menentukan kemungkinan prediksi t(8;21)(q22;q22) atau fusi gen AML-ETO(MTG8) pada LMA. Kesemua kasus merupakan kasus AML-M2. Kami menemukan bahwa ciri khas morfologis yang mengindikasikan adanya t(8;21) dan membedakannya dengan kasus AML-M2 tanpa t(8;21) adalah susbtansi sitoplasma kemerahan pada netrofil (75%), mielosit netrofilik atau metamielosit tanpa granula atau dengan sedikit granula (2,3%), eosinofilia (mielosit dan metamielosit eosinofilik) (>5%), mielosit dengan banyak granula (8.5%), dan blast tipe I dalam persentase rendah (<10%). Ciri khas ini tidak terdapat pada kasus M2 tanpa t(8;21) atau AML1-ETO(MTG8). Adanya mielosit dengan banyak granula yang kami laporkan ini belum pernah dilaporkan sebelumnya. Ciri khas lainnya sangat sesuai dengan yang dilaporkan sebelumnya oleh Nakamura et al. (**Med J Indones 2007; 16:84-8**)

Abstract

The laboratory systems for chromosomal analysis or the detection of fusion genes are generally not available in Indonesia. Therefore, bone marrow (BM) morphological analysis should be developed and applied to get an accurate diagnosis. In this study the BM smears of eight (8) cases of acute myeloblastic leukemia (AML) which had already been known to have t(8;21)(q22;q22), were morphologically evaluated in order to find out the characteristics, which might be used to predict t(8;21)(q22;q22) or the presence of AML1-ETO(MTG8) fusion gene. All of the cases belonged to AML-M2. The morphological characteristics, indicative of t(8;21) were pink colored cytoplasm in mature neutrophil (75%), neutrophilic myelocytes or metamyelocytes without granules or with scarce granules (2.3%), eosinophilia (eosinophilic myelocytes and metamyelocytes) (above 5%), myelocytes with abundant granules 8.5%, and low percentage of type I blasts (below 10%). These characteristics were not observed in AML-M2 cases without t(8;21) or AML1-ETO(MTG8). The myelocytes with abundant granules have not been described so far, while other characteristics were in line with the findings of Nakamura et al (Leukemia 1997;11:651-55). (**Med J Indones 2007; 16:84-8**)

Keywords: PML-RARA fusion gene, AML1-ETO (MTG8), myelocytes, abundant granul

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Acute myeloid leukemia (AML) constitutes a heterogeneous group of leukemic disorder with diverse biologic and clinical features. Traditionally, AML was first sub-classified by morphology and cytochemistry in the French-American-British (FAB) classification system.[1] However, over the past three decades, the diagnosis of subtypes of AML and the therapeutic measures have been remarkably improved. Such diagnostic systems include karyotypic analysis, molecular genomic analysis, and phenotypic analysis such as the flow cytometric analysis of cell surface antigens.

Recurrent chromosomal aberrations have been extensively described in leukemia and lymphoma, and some of

them are regarded as the primary gene abnormality causing a certain type of leukemia. Translocation t(15;17)(q22;q21) or PML-RARA fusion gene was detected in more than 90% of M3 cases.[2,3] However, less common M3 cases with t(11;17)(q23;q21), or unbalanced der(5) t(5;17)(q13;q21) have been reported.[4,5] It seems that the disruption of RARA gene, leading to the fusion gene formation between RARA and some other gene, is the primary cause in M3. Thus, the relationship between the primary gene abnormality and the FAB diagnosis is remarkably specific, although exceptionally rare immature morphology has been reported.[6,7] Compared with M3, research results concerning the relationship between AML-M2 and t(8;21)(q22;q22) were inconclusive. In a study, AML-M2 with t(8;21) constituted 20% of all M2 cases, while in another study approximately 90% of the AML cells with t(8;21) show M2 morphology.[8,9] While a very few reports have been published regarding the morphological characteristics in AML with t(8;21), it is unknown how widely such information is being used in actual hematology laboratories to forecast t(8;21)(q22;q22) or AML1-ETO (MTG8).

The objective of this study was to investigate the morphological characteristics of bone marrow aspirate smears of AML-M2 cases with t(8;21), since this type of information is practically meaningful in Indonesia where chromosomal analysis or RT-PCR analysis is generally unavailable for the time being. Informations concerning morphological characteristics might be used to predict t(8;21) (q22;q22) or the presence of AML1-ETO(MTG8) fusion gene.

METHODS

We studied morphological characteristics of bone marrow (BM) aspirate smears retrospectively from 8 patients with AML M2 associated with t(8;21)(q22;q22) which were diagnosed in the Department of Laboratory Medicine, Kobe University Hospital, Kakogawa City Hospital, and Kobe Central City Hospital between January 2003 - August 2005. Bone marrow smears were done and collected since the time of diagnosis. The AML-M2 was diagnosed according to the FAB classification, and cytogenetic analysis for t (8;21)

(q22;22) were performed by G-banding at the time of diagnosis in those hospitals.

The smears were evaluated by the FAB classification.[1] Type I, Type II and Type III myeloblasts were determined according to the FAB classification. Type I blasts lack cytoplasmic granules, but possess prominent nucleoli, a central nucleus, and uncondensed chromatin patterns. Type II blasts retain a central nucleolus but have a few primary azurophilic cytoplasmic granules. Type III blasts have more than 20 azurophilic cytoplasmic granules with the characteristics of promyelocytes.[10] The leukemia cells with Auer bodies were counted among the blasts cells.

Immunophenotyping were done on bone marrow samples of those patients by flowcytometry at the time of diagnosis.

Cell surface antigen analysed were CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD13, CD14, CD33, CD34, CD56, and HLADR that were tested in commercial laboratories.

The AML1-ETO (MTG8) fusion RNA was analyzed by reverse transcription (RT)-PCR in commercial laboratories. The data of immunophenotyping, cell surface antigen and AML1-ETO (MTG8) fusion RNA were collected from medical records.

The data of morphological analysis were tabulated according to percentage of each cell morphology, and data of cell surface antigen analysis were tabulated for positive and negative results.

RESULTS

The morphological analysis is shown in Table 1. The results of the cell-surface antigen analysis, which were available in 5 of 8 cases studied, are shown in Table 2. The CD13 and CD33, both of which belong to the myeloid and AML-related antigen, CD 19, a B-lineage antigen, CD34, a stem cell antigen, and HLA-DR were expressed in all cases, while CD56 was detected in 4 of the 5 cases. The AML1-ETO (MTG-8) fusion RNA was detected in all cases.

Table 1. Morphological analysis of t(8;21)(q22;q22)

Number of Cases:	1 (%)	2 (%)	3 (%)	4 (%)	5 (%	6 (%	7 (%)	8 (%	Mean (%)
Morphology:									
Myeloblasts type I	5.2	8	28.5	9	4	5	8	6	9.2
Myeloblasts type II	5.6	9	13.5	8	12	7.5	8	5	8.5
Myeloblasts type III	20.8	24	4	35	44	49	35	35	30,8
Total Myeloblasts	31,6	41	46	52	60	61,5	51	46	48,6
Promyelocytes	12	11	11	8	8	10	5	8	9.1
Myelocytes with scarce granules	0,6	1.5	2	1.3	1.5	0.5	5	5	2.1
Myelocytes G +	13	11.5	2.5	9	6.5	8	9	9	8,5
Metamyelocytes with scarce granules	2	1.5	2	1.3	4	2	3	3	2.3
Metamyelocytes G+	9,6	11	2	3.3	5	5	9	6	6.3
Eosinophilia	14,4	7	2	7	6	5	6	7	6,8
Bands	5,6	2	2.5	3	2	2	4	5	3.7
Segmented G	7,2	5	9	2	2	3	5	5	4.7
Erythroblast	4	8.5	21	13	5	3	3	6	7.9
Megakaryocytes	0	0	0	0	0.4	0	0	0.1	0.06
Auer Rods	7	4	2	11.5	0	1	0.5	4.5	3.8
MPO	11	9	NA	NA	4	5	NA	NA	6.3
Pink colored cytoplasm in mature neutrophils	79.2	85.2	25.8	84.5	85.4	84.8	76.3	78.9	75.0

(MPO : myeloperoxidase;   NA = not available; Myelocytes G+= myelocytes with abundant granules; Metamyelocytes G+ = metamyelocytes with abundant granules; Segmented G = segmented granulocytes)

Table 2. The results of cell-surface antigen analysis of t(8;21)(q22;q22)

Patient code	Age	Sex	CD13	CD19	CD33	CD34	CD56	CD71	CD117	HLADR
K	45	F	+	+	NA	+	+	+	+	+
N	6	M	+(72.1%)	+(49%)	+(11.1%)	+(94.9%)	-	-	-	+(98%)
I	18	F	+(25.4%)	+(12.9%)	+(20%)	+(62.4%)	+(14.6%)	-	-	+(98%)
W	58	F	+(20.4%)	+(78%)	+(33%)	+(64.2%)	+(99%)	-	-	+(98%)
O	54	M	+(31.8%)	+(19%)	+(55%)	+(67.6%)	+(98.8%)	-	-	+(99.3%)

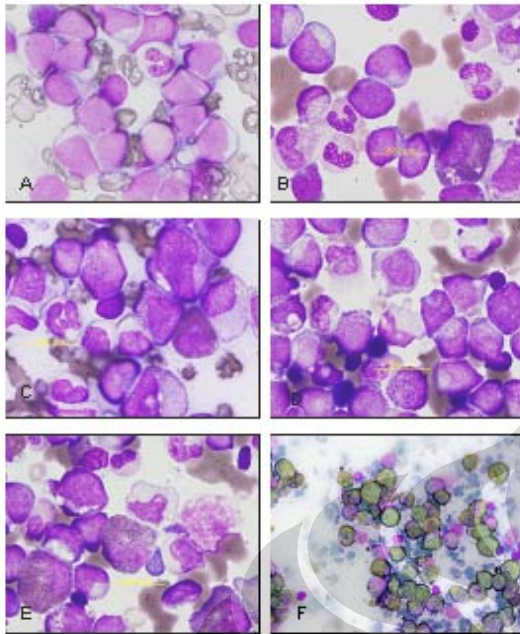F = female, M = male, NA = not available, +( ) = % tage of positive results, + = positive, - = negative

*Figure 1. Specimens taken from t(8;21)⁻ and t(8;21)⁺ cases
Panel A: a specimen from a t(8;21)⁻ case showed mature
neutrophils with pale colored cytoplasm without any granules
and myeloblasts type I, panel B: mature neutrophils from a
t(8;21)⁺ case showing homogenous pink colored cytoplasm,
panel C: the blast cells type III from a t(8;21)⁺ case, panel D:
myelocytes with abundant granules from a t(8;21)⁺ case, panel
E: eosinophils with abundant granules, from a t(8;21)⁺ case, and
panel F: myeloperoxidase reaction from a t(8;21)⁺ case.*

DISCUSSION

While two reports have described the morphologic
features for AML-M2 with t(8;21)[11,12] there have been
no such description in the monographs or textbooks of
hematology.[13-16] Therefore, the reliability of the
morphological characteristics to forecast t(8;21) is not
known .

Jaffe et al reported several common morphological
features in AML with t(8;21), but these features are
not specific. In our attempt to diagnose AML-M2
patients with t(8;21) based on the morphological
characteristics, we found pink colored cytoplasm in
most of mature neutrophils, myelocytes with scarce
granules, myelocytes with abundant granules,
eosinophilia (eosinophilic myelocytes or metamyelocytes above
5%) very low percentage of type I blasts and a high
percentage of type III blasts. These findings were not
found in any of BM smears from AML-M2 patients
without t(8;21)(q22;q22) (unpublished result). Among

these characteristics, myelocytes with abundant granules
have not been reported yet, and supposed to be a new
finding which could characterize AML-M2 with
t(8;21) specifically.

Nakamura et al compared 30 AML-M2 with t(8;21)
with 50 AML-M2 without t(8;21). It was disclosed
that irregular nuclear shape, Auer bodies and
homogeneous pink colored cytoplasm of mature
neutrophils were observed in 90 to 100% of the AML
with t(8;21). Pink colored cytoplasm were very
characteristic. Conversely, pale-colored cytoplasm
without any granules was observed in 84% of the
AML-M2 without t(8;21)(q22;q22).[12] Thus, most of
our observations, except for "myelocytes with abundant
granules", confirmed the findings of Nakamura et al.

CD13, CD19, CD33, CD34, and HLA-DR were
expressed by the leukemia cells from all 5 tested
patients, while CD56 was expressed in 4 of 5 patients.
We found that the incidence of expression of CD34
and HLA-DR was quite high. This finding was in line
with other findings which showed that the incidence
of the expression of CD34 and HLA-DR in t(8;21)
AML cells was significantly higher than those in
AML-M2 without t(8;21).[17,18] Borowitz et al (1989)
have reported a high positivity of CD34 (45%) in the
more immature leukemias,[19] and Ferrara et al 1998
reported that t(8;21) showed significant high expressions
of CD19,CD34, CD56, CD45RA and CD54.[20]

The CD19 antigen, which is usually a B-lineage specific
antigen, has been known as very characteristic for
AML with t(8;21).[18] This observation seems to be
associated with the absence of CD7 antigen expression.[21]
The high incidence of CD34 expression and the
lineage promiscuity such as in those showing CD19
antigen expression suggest that this translocation
occurs during early stage of hematopoietic differentiation.[18]

Some authors reported that CD56 expression in
t(8;21) AML was significantly associated with shorter
complete remission (CR) and survival rates.[22] The
expression of CD56 is usually restricted to the
lymphoid malignancies of T/ natural killer (NK)-cell
origin. In AML, CD56 expression was detected in
20% of cases, and was generally associated with
cytogenetic abnormalities such as t(8;21) or trisomy.[23]
The fact that CD56 antigen is a neural cell adhesion
molecule, is implicated in the observation that
extramedullary tumor formation is most frequently
found in AML with t(8;21).[23,24]

When t(8;21)(q22;q22) is suspected morphologically, the preservation of frozen samples or metaphase spread is strongly recommended to confirm the diagnosis. By using the frozen sample, the later reverse transcription (RT) PCR (RT-PCR) can indicate AML-1-ETO(MTG8) fusion gene. As long as suitable metaphase spreads are available, a later chromosomal analysis can confirm t(8;21)(q22;q22). The awareness of the various morphological characteristics for t(8;21) can keep hematologists being aware of the subclasses of AML, and can promote the use of the above laboratory procedures.

In conclusion, the characteristic of AML-M2 with t(8;21)(q22;q22) are pink colored cytoplasm in mature neutrophils, neutrophilic myelocytes or metamyelocytes without granules or with scarce granules, eosinophilia (eosinophilic myelocytes and metamyelocytes), myelocytes with abundant granules and low percentage of type I blasts.

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