WT1 mRNA expression (copies/μg RNA) = (measured WT1 mRNA [copies/mL]/measured GAPDH mRNA [copies/mL]) × 2.7 × 10^7 (copies/μg RNA)

PB cut-off value

The lower limit of the WT1 mRNA measurement range in the WT1 assay kit is 2500 copies/mL, or 50 copies/μg RNA when converted to copies per microgram of RNA. In this study, a value of 50 copies/μg RNA was set as the cut-off value for WT1 mRNA expression, and a value of 50 or more copies/μg RNA was judged as positive according to the instruction manual of the WT1 mRNA assay kit.

Statistical analysis

The mean ± SD for the log-transformed values of WT1 mRNA expression (copies/μg RNA) was calculated, and then converted back to base 10 and used as the geometric mean. All data below the detection limit were shown as 49 copies/μg RNA. For intergroup comparison of WT1 mRNA expression, a Tukey-Kramer honestly significant difference (HSD) test was performed at the level of significance of $p < 0.05$ using log-transformed values of WT1 mRNA expression (copies/μg RNA). For comparison of WT1 mRNA expression between the aplastic anemia (AA) and RA groups, a Wilcoxon rank-sum test and Steel test were performed at the level of significance of $p < 0.05$ using log-transformed values of WT1 mRNA expression (copies/μg RNA). The Pearson correlation coefficient was used for analysis of each correlation.

Results

As a result of the central review conducted on all 172 patients, 115 were classified as patients with MDS in the FAB classification, excluding chronic myelomonocytic leukemia (CMML). Similarly, 98 patients in the 2001 WHO classification and 97 in the 2008 WHO classification were classified as patients with MDS (Figure 1).

Analytical results based on FAB classification

WT1 mRNA expression in PB and BM

The 172 patients eligible for analysis were categorized by disease type, and their WT1 mRNA expression levels in PB and BM are shown in Table I. The mean WT1 mRNA expression level in the 115 patients with MDS (excluding CMML) was 360 copies/μg RNA in PB and 2240 copies/μg RNA in BM, and these values were the second highest after the values obtained in patients with AML-MDS (PB: 12 600 copies/μg RNA; BM: 33 100 copies/μg RNA). On the other hand, the WT1 mRNA expression level was less than 50 copies/μg RNA in PB and 90-630 copies/μg RNA in BM in patients with AA, idiopathic cytopenia of unknown significance (ICUS), idiopathic thrombocytopenic purpura (ITP), paroxysmal nocturnal hemoglobinuria (PNH), pure red-cell aplasia (PRCA) and erythroid hypoplasia, which were all lower compared with the level in MDS.

The relationship between WT1 mRNA expression in PB and BM was evaluated in all patients. The regression line formula $y = 0.7329x + 1.4407$ was obtained, indicating a strong correlation ($r = 0.85$) (Figure 2).

WT1 mRNA expression in PB and BM for each MDS disease stage

When the WT1 mRNA expression levels in PB and BM were compared for each MDS subtype based on the FAB classification [Figure 3(a)], the level in both increased proportionally with each MDS classification as the disease

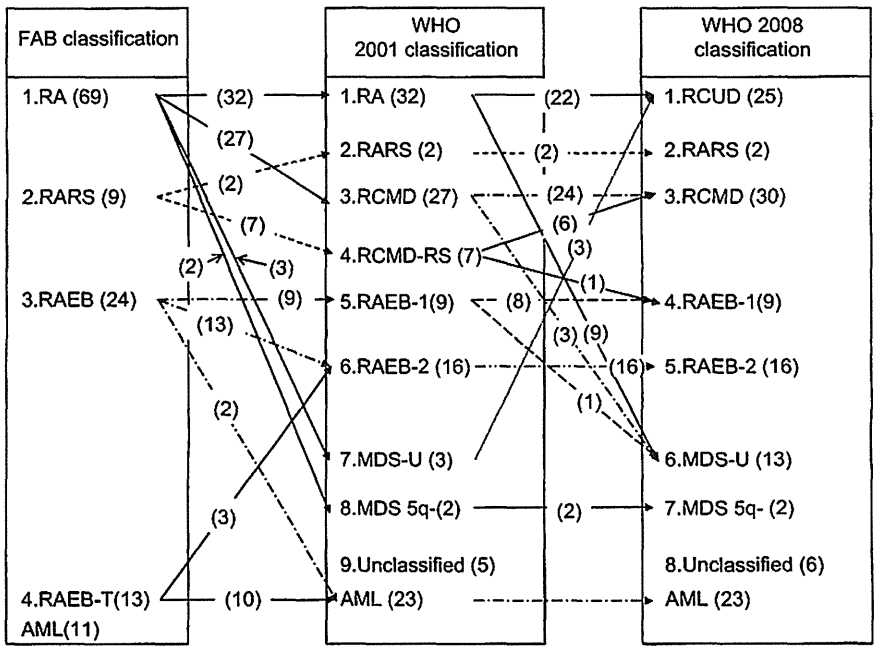

Figure 1. FAB and WHO classification of myelodysplastic syndromes in this study. FAB classification-based MDS subtypes (four subtypes: RA, RARS, RAEB and RAEB-t), 2001 WHO-based MDS subtypes (eight subtypes: RA, RARS, RCMD, RCMD-RS, RAEB-1, RAEB-2, MDS-U and MDS 5q−), 2008 WHO-based MDS subtypes (seven subtypes: RCUD, RARS, RCMD, RAEB-1, RAEB-2, MDS-U and MDS 5q−). Numbers in parentheses represent numbers of patients.

Table I. WT1 mRNA expression levels in PB and BM from patients with different MDS subtypes and AML-MDS according to FAB classification.

		WT1 mRNA expression level			
		Peripheral blood		Bone marrow	
Disease	No. of patients	Log (mean ± SD)	Geometric mean (copies/μg RNA)	Log (mean ± SD)	Geometric mean (copies/μg RNA)
MDS	115	2.56 ± 1.05	360	3.35 ± 0.87	2240
AML-MDS	11	4.10 ± 0.96	12 600	4.52 ± 0.77	33 100
AML-MDS (CR)	2	1.89 ± 0.20	80	2.98 ± 0.39	1000
CMML	3	2.17 ± 0.54	150	3.04 ± 0.54	1100
CLL	1	1.92	80	3.33	2140
Atypical CML	1	—	<50	1.95	90
AA	8	—	<50	2.64 ± 0.37	440
ICUS	3	—	<50	2.16 ± 0.36	140
ITP	1	—	<50	2.13	130
PNH	1	—	<50	2.8	630
PRCA	2	—	<50	2.17 ± 0.12	150
Erythroid hypoplasia	1	—	<50	1.94	90
Unclassified	23	2.14 ± 0.56	140	2.96 ± 0.61	910
Total	172	2.50 ± 1.05	320	3.27 ± 0.90	1860

PB, peripheral blood; BM, bone marrow; MDS, myelodysplastic syndromes; AML-MDS, acute myeloid leukemia-evolved MDS; FAB, French-American-British; CR, complete remission; CMML, chronic myelomonocytic leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; AA, aplastic anemia; ICUS, idiopathic cytopenia of unknown significance; ITP, idiopathic thrombocytopenic purpura; PNH, paroxysmal nocturnal hemoglobinuria; PRCA, pure red-cell aplasia.

stage progressed. Significant differences in both PB and BM expression were seen between RA and RAEB, RA and RAEB-t, refractory anemia with ringed sideroblasts (RARS) and RAEB, and RARS and RAEB-t ($p < 0.05$).

WT1 mRNA expression in PB and BM for each IPSS risk group

WT1 mRNA expression levels in PB and BM for each IPSS risk group were compared in the 115 patients with MDS. A tendency for WT1 mRNA expression to increase in both PB and BM was observed in each IPSS risk group as the risk of transformation to AML increased from low to high. Significant differences ($p < 0.05$) in WT1 mRNA expression were observed in risk groups between low and intermediate-2, low and high, intermediate-1 and intermediate-2, and intermediate-1 and high in PB samples; and between low and intermediate-1, low and intermediate-2, low and high, intermediate-1 and intermediate-2, and intermediate-1 and higher in BM samples [Figure 3(b)]. The correlation between IPSS score and WT1 mRNA expression was evaluated, and a correlation of $r = 0.57$ was found for both PB and BM samples.

Next, the WT1 mRNA expression levels in PB and BM between IPSS risk groups were compared in the 69 patients with RA [Figure 3(c)]. As the risk increased from low to intermediate-2, the level of WT1 mRNA expression in both PB and BM increased. Moreover, when the distribution of WT1 mRNA expression between each risk group was evaluated, a significant difference ($p < 0.05$) was found in PB between low and intermediate-2; in BM, significant differences were found between low and intermediate-1, and low and intermediate-2.

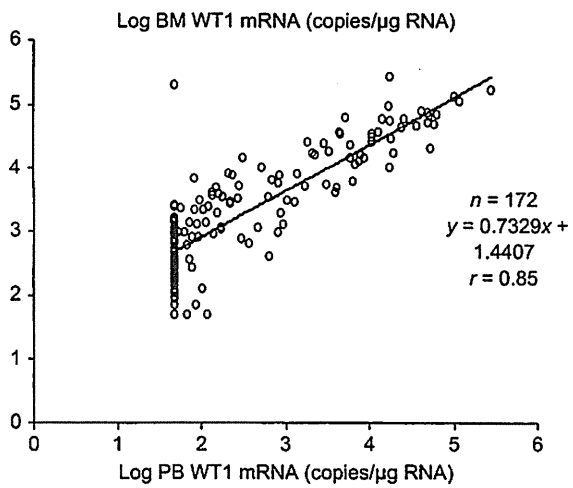

Figure 2. Correlation of WT1 mRNA expression in PB and WT1 mRNA expression in BM.

Correlation between IPSS karyotype and WT1 mRNA expression

A total of 114 patients with MDS were categorized into the three prognostic groups of good, intermediate and poor in accordance with their IPSS karyotype, and the levels of WT1 mRNA expression in their PB and BM samples were compared. One patient with MDS was excluded from this analysis because chromosome testing was not performed. The WT1 mRNA expression level increased in both PB and BM samples as the karyotype indicated a poorer prognosis. Among karyotypes, significant differences ($p < 0.05$) in WT1 mRNA expression were found between the good and intermediate and between the good and poor groups [Figure 3(d)].

Correlation between WT1 mRNA expression and percentage of blasts in BM

The correlation between blast ratio and WT1 mRNA expression in PB and BM was investigated in 114 patients with MDS (excluding one patient in whom the blast ratio could not be measured). The correlation between blast ratio and PB WT1 mRNA expression was $r = 0.51$, and the correlation between blast ratio and BM WT1 mRNA expression was $r = 0.48$.

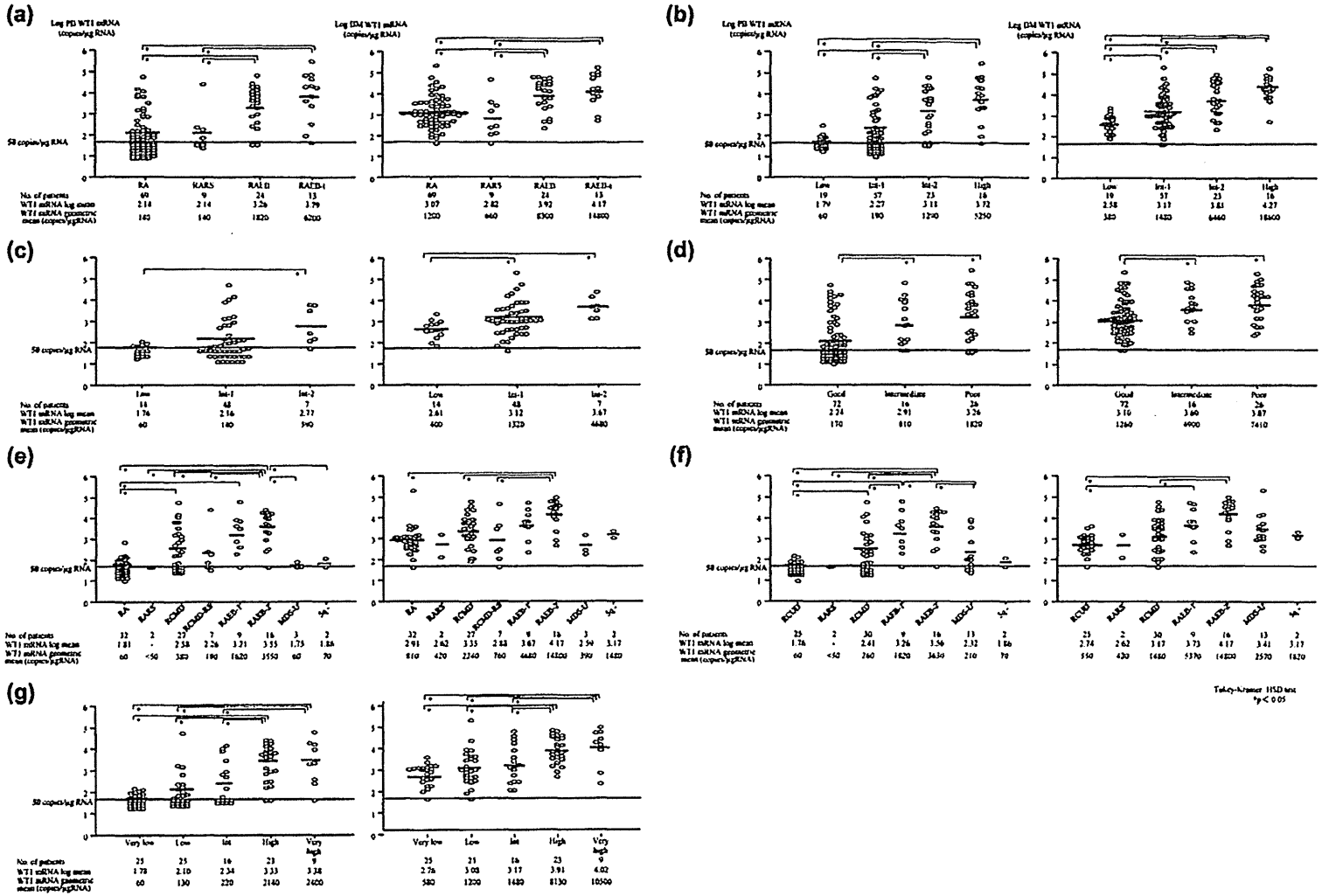

Figure 3. WT1 mRNA expression levels in PB and BM from patients with MDS (a) according to FAB classification, (b) according to IPSS category, (c) patients with RA according to IPSS category, (d) according to chromosomal karyotype, (e) according to WHO 2001 classification, (f) according to WHO 2008 classification, (g) according to WPSS category. In intergroup comparison of WT1 mRNA expression, the Tukey-Kramer HSD test was performed using log-transformed values of WT1 mRNA expression with a level of significance of $p < 0.05$. Bold lines represent mean WT1 mRNA expression after log transformation. Fine lines represent lower limit of detection of WT1 mRNA (50 copies/μg RNA).

Analytical results based on 2001 WHO classification

WT1 mRNA expression in PB and BM for each MDS disease stage based on 2001 WHO classification

Figure 3(e) shows the assay results for WT1 mRNA expression in PB and BM in 98 patients in various MDS disease stages categorized on the basis of the 2001 WHO classification. The WT1 mRNA expression levels in both PB and BM tended to increase with the progression to each MDS subtype. When the levels of WT1 mRNA expression in each disease stage were investigated, significant differences ($p < 0.05$) were found in PB between RA and refractory cytopenia with multilineage dysplasia (RCMD), RA and RAEB-1, RA and RAEB-2, RARS and RAEB-2, RCMD and RAEB-2, RCMD with ringed sideroblasts (RCMD-RS) and RAEB-2, RAEB-2 and unclassified MDS (MDS-U), and RAEB-2 and 5q− syndrome; in BM, significant differences were found between RA and RAEB-2, RCMD and RAEB-2, and RCMD-RS and RAEB-2.

Correlation between WT1 mRNA expression and percentage of blasts in BM based on 2001 WHO Classification

The correlation between the blast ratio and WT1 mRNA expression in PB and BM was investigated in 97 patients with MDS (excluding one patient in whom the blast ratio could not be measured). The correlations between the blast ratio and WT1 mRNA expression were $r = 0.50$ in PB and $r = 0.46$ in BM.

Analytical results based on 2008 WHO classification

WT1 mRNA expression in PB and BM for each MDS disease stage based on 2008 WHO classification

Figure 3(f) shows the assay results for WT1 mRNA expression in PB and BM in a total of 97 patients in various MDS disease stages categorized on the basis of the 2008 WHO classification. WT1 mRNA expression in both PB and BM tended to increase with the progression to each MDS subtype.

When the distribution of WT1 mRNA expression for each disease stage was examined, significant differences ($p < 0.05$) were found in PB between refractory cytopenia with unilineage dysplasia (RCUD) and RCMD, RCUD and RAEB-1, RCUD and RAEB-2, RARS and RAEB-2, RCMD and RAEB-1, RCMD and RAEB-2, RAEB-1 and MDS-U, and RAEB-2 and MDS-U; in BM, significant differences were found between RCUD and RAEB-1, RCUD and RAEB-2, and RCMD and RAEB-2.

Correlation between WT1 mRNA expression and percentage of blasts in BM based on 2008 WHO classification

The correlations between blast ratio and WT1 mRNA expression in 96 patients (excluding one patient with MDS whose blast ratio could not be measured) were $r = 0.50$ in PB and $r = 0.46$ in BM.

WT1 mRNA expression in PB and BM for each WPSS risk group

WT1 mRNA expression in PB and BM was compared in 98 patients with MDS classified according to WPSS risk group [Figure 3(g)]. As the risk increased from very low to very high, WT1 mRNA expression in both PB and BM also tended to rise. When the distribution of WT1 mRNA for each risk group was evaluated, significant differences ($p < 0.05$) were found in both PB and BM between very low and high, very low and very high, low and high, low and very high, intermediate and high, and intermediate and very high. Moreover, when the correlation between the WPSS score and WT1 mRNA expression was investigated, the values were $r = 0.61$ in PB and $r = 0.55$ in BM.

Differential diagnosis between RA and AA

Differential diagnosis based on WT1 mRNA expression in PB samples

The WT1 mRNA expression level in PB was less than 50 copies/μg RNA in all eight patients with AA, whereas it was less than 50 copies/μg RNA in 34 patients with RA and 50–52 100 copies/μg RNA in 35 of 69 patients with RA. The statistical analysis by Wilcoxon rank-sum test revealed a statistical difference between eight patients with AA and 65 patients with RA ($p = 0.01$). Sixty-nine patients with RA were further categorized into three groups by bone marrow findings: hypoplastic RA ($n = 20$), hyperplastic RA ($n = 15$) and normoplastic RA ($n = 30$), excluding the non-categorized RA ($n = 4$). Significant differences were observed between AA and each of hypoplastic ($p = 0.04$) or normoplastic RA ($p = 0.02$), whereas no difference was shown between the AA and hyperplastic RA group ($p = 0.10$) by Steel test (Figure 4). From these findings, a differential diagnostic cut-off value between RA and AA of 50 copies/μg RNA for WT1 mRNA expression in PB is considered appropriate, for which the sensitivity was 50.7% (35/69) and the specificity was 100% (8/8).

Differential diagnosis based on WT1 mRNA expression in BM samples

The WT1 mRNA expression level in BM was 251–2600 copies/μg RNA in eight patients with AA, whereas it was less than 50 copies/μg RNA in one of 69 patients with RA and 69–196 000 copies/μg RNA in the others. The statistical analysis by Wilcoxon rank-sum test revealed no statistical difference between eight patients with AA and 65 patients with RA. Sixty-nine patients with RA were similarly categorized into three groups: hypoplastic, hyperplastic and normoplastic RA, excluding the non-categorized RA. Statistical analysis by Steel test revealed a significant difference between AA and normoplastic RA groups ($p = 0.04$), whereas there were no significant differences between the AA and each of hypoplastic RA and hyperplastic RA groups (Figure 4).

When receiver operating characteristic (ROC) analysis was performed to evaluate the performance of BM WT1 mRNA expression as an indicator to differentiate between RA and AA, the area under the curve was 0.713, and the Youden index [18] showed 432 copies/μg RNA. Moreover, the sensitivity was 69.6% (48/69), and the specificity was 75.0% (6/8) (Supplementary Figure to be found online at http://informahealthcare.com/doi/abs/10.3109/10428194.2012.745074).

When the PB cut-off value of 50 copies/μg RNA was inserted into the regression line formula obtained

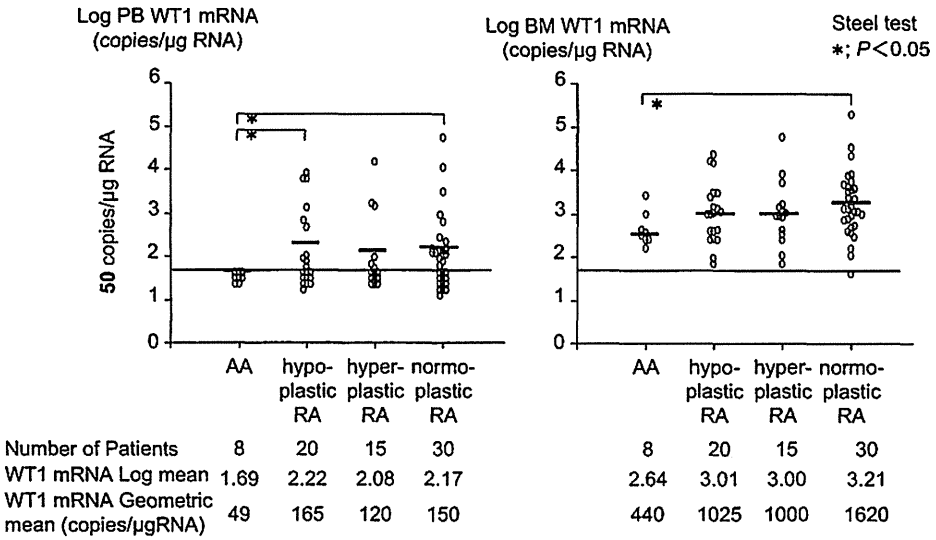

Figure 4. Comparison of WT1 mRNA expression between AA and RA groups (hypoplastic, hyperplastic and normoplastic RA). In intergroup comparison of WT1 mRNA expression, Steel test was performed using log-transformed values of WT1 mRNA expression with a level of significance of $p < 0.05$. Bold lines represent mean WT1 mRNA expression after log transformation. Fine lines represent lower limit of detection of WT1 mRNA (50 copies/μg RNA).

from the correlation between WT1 mRNA expression in PB and BM (Figure 2), BM WT1 mRNA expression became 480 copies/μg RNA. When 500 copies/μg was evaluated as the cut-off value for BM WT1 mRNA expression, the sensitivity was 68.1% (47/69) and the specificity was 75.0% (6/8). Based on these results, 500 copies/μg RNA was considered to be an appropriate cut-off value for the differential diagnosis between RA and AA using WT1 mRNA expression in BM.

Comprehensive analysis using cut-off values

The PB and BM samples in each disease and MDS subtype were further evaluated for their WT1-positive rates, using the WT1 mRNA expression cut-off values determined above (PB: 50 copies/μg RNA; BM: 500 copies/μg RNA) (Table II). For AML-MDS (11 patients), the WT1 mRNA-positive rates were a high 100% (11/11) for PB and 90.9% (10/11) for BM, and in MDS (115 patients), the WT1 mRNA-positive rates were 61.7% (71/115) for PB and 73.0% (84/115) for BM, which were the second highest after AML-MDS. In contrast, all patients with AA, ICUS, ITP, PNH, PRCA and erythroid hypoplasia had low positive rates of 0% for PB and 18.8% (3/16) for BM. The WT1 mRNA-positive rates for PB and BM increased with MDS disease stage progression (Table II).

Table II. WT1 mRNA-positive rate in PB and BM from patients with different MDS subtypes and AML-MDS according to FAB classification.

		WT1 mRNA-positive rate (%)	
Subtype	No. of patients	Peripheral blood	Bone marrow
RA	69	50.7 (35/69)	68.1 (47/69)
RARS	9	44.4 (4/9)	44.4 (4/9)
RAEB	24	83.3 (20/24)	87.5 (21/24)
RAEB-t	13	92.3 (12/13)	92.3 (12/13)
AML-MDS	11	100.0 (11/11)	90.9 (10/11)
Total	126	65.1 (82/126)	74.6 (94/126)

PB, peripheral blood; BM, bone marrow; MDS, myelodysplastic syndromes; AML-MDS, acute myeloid leukemia-evolved MDS; FAB, French-American-British; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RAEB, refractory anemia with excess of blasts; RAEB-t, refractory anemia with excess of blasts in transformation.

Discussion

In this study, the clinical usefulness of the measurement of WT1 mRNA expression in risk assessment of MDS was evaluated using a WT1 assay kit. Recently, a steady stream of reports has indicated the usefulness of WT1 mRNA measurement. The group of Cilloni [6] confirmed that WT1 mRNA expression potentially fulfills all the requirements for an additional marker for risk assessment in MDS, compared with the conventional methods. The measurement of WT1 can be effective, particularly in cases in which BM aspiration and/or cytogenetic analysis fail or are not informative [6].

Furthermore, in their findings in a long-term prospective study, Tamura *et al.* [19] reported that a significant correlation ($p = 0.0186$) was seen between WT1 mRNA expression and survival time when WT1 mRNA expression in PB was categorized into three groups of less than 10^2, 10^2–10^4, and greater than 10^4 copies/μg RNA, that the median survival time for each group was 62.7 months, 29.9 months and 11.6 months, respectively; and that the time until transformation to leukemia was the shortest in the group with the highest WT1 mRNA expression. In addition, they reported that in univariate analysis, WT1 mRNA expression was a predictive parameter for transformation to leukemia, and in multivariate analysis, it was a significant predictive parameter along with the IPSS score [19]. As described above, Tamaki *et al.* reported similar findings [4].

This study was conducted using not only the FAB classification system but also the 2001 and 2008 WHO classification systems. It was confirmed that in all three classification systems, WT1 mRNA expression in both PB and BM increases significantly in MDS subtypes with disease stage

progression. In addition, both PB and BM WT1 mRNA expression increased significantly as the risk of transformation to AML rose in the IPSS and WPSS risk groups. Furthermore, a correlation of $r = 0.57$ between the IPSS score and WT1 mRNA expression was seen in both PB and BM. The correlations between the WPSS score and WT1 mRNA expression were $r = 0.61$ in PB and $r = 0.55$ in BM. In comparison with the IPSS, the WPSS allows the assessment of survival time and progression of leukemic transformation at all time periods during the clinical course, leading to continued prognostic evaluation while reviewing the risk. WT1 mRNA expression correlates with the WPSS prognosis, and despite the single-point quantitation, the results in this study indicate that WT1 mRNA is useful as a time-course prognostic marker in the same manner as the WPSS.

At present, allogeneic hematopoietic stem cell transplant is the only curative treatment for MDS. However, determination of the timing of allogeneic transplant is very difficult because many patients are older, treatment-related deaths frequently occur, and there are large individual differences in the rate of disease progression. Allogeneic transplant is selected as the therapeutic regimen for MDS when no increase in blast cells is confirmed, taking into consideration the development of transfusion dependency and frequency of infections [20]. In addition, allogeneic transplant is selected when a future increase in blast cells is predicted by karyotypic analysis even though no increase is currently observed. It is recommended that transplant be performed before the progression to cytopenia caused by an increase in blast cell clones and before the progression to acute leukemia, although induction chemotherapy may be required when an increase in blast cells is observed [21]. On the other hand, another study suggested that delaying transplant until the advanced stage of disease results in a longer survival time for low and intermediate-1 IPSS risk groups, while early transplant was recommended for the intermediate-2 and high groups [22]. The period after CR is achieved is considered to be the standard timing to perform transplant for acute leukemia, but determining CR is extremely challenging. Our results revealed that periodic monitoring of WT1 mRNA expression in patients with MDS provided useful information for predicting the timing of transplant.

RA, a subtype in the early MDS disease stage, is often difficult to differentiate from AA [23]. In a previous study by Iwasaki *et al.*, no difference in WT1 mRNA expression was observed between RA and AA [9]. However, our data revealed the possibility of WT1 expression level to differentiate AA and RA groups using both peripheral blood and bone marrow samples (Figure 4). In the present statistical analysis, significant differences were observed between AA and hypoplastic RA ($p = 0.04$) in PB. The number of subjects was limited, and further trial is required for more detailed analysis. Moreover, tentative cut-off values for WT1 mRNA expression were set at 50 copies/μg RNA in PB and 500 copies/μg RNA in BM. Although the number of patients was small, the results showed that the level of WT1 mRNA expression could differentiate between RA and AA, with specificity in PB and BM of 100% (8/8) and 75.0% (6/8), respectively. This provides evidence that the measurement of WT1 mRNA expression can play a role in the differential diagnosis of RA and AA.

The WT1 assay kit is used clinically in Japan as a marker to monitor MRD in patients wth AML. In MDS, a clonal disorder of pluripotent hematopoietic stem cells, WT1 mRNA expression increases depending on the MDS subtype and disease stage. In contrast, the mechanism by which WT1 mRNA expression increases in MDS is not considered to correlate simply with the fluctuation in leukemic clones, as seen in AML. In normal hematopoiesis, WT1 mRNA is expressed mainly in CD34-positive cells. In contrast, in patients with MDS, WT1 mRNA is also expressed in CD34-negative cells, particularly in lineages exhibiting abnormalities [24]. In our study, the level of WT1 mRNA expression within the RA group was shown to increase with the increase in IPSS risk [Figure 3(c)]. Moreover, a similar trend of increasing WT1 expression was found in the RCUD and RCMD groups according to the 2008 WHO classification, although no significant increase in blast cells in BM was observed in these groups. Taken together, these findings indicate that the increase in WT1 mRNA expression in patients with MDS may reflect the divergence of MDS clones from normal clones and preleukemic changes.

In patients with MDS, evaluating the changes in WT1 mRNA levels simultaneously in PB and BM samples provides useful information on disease stage progression or risk assessment in individual patients. In addition, the WT1 mRNA-positive rate in each subtype of MDS was high (50–90%) in both PB and BM in this study, suggesting that a single measurement of WT1 mRNA is sufficient for MDS diagnosis, particularly for differentiating RA from AA.

Overall, this study provides evidence that the measurement of the level of WT1 mRNA expression in PB and BM serves as a supplemental marker for MDS diagnosis and prognostic assessment. This assay has great potential to contribute to more appropriate diagnoses and therapeutic decisions in patients with MDS and to evaluate the timing of allogeneic transplant.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal.

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Supplementary material available online

Supplementary figure showing ROC analysis of WT1 mRNA expression in BM in RA and AA groups

CME/SAM

Correlation Between Dysplastic Lineage and Type of Cytopenia in Myelodysplastic Syndromes Patients With Refractory Anemia According to the FAB Classification

Akira Matsuda, MD,[1] Itsuro Jinnai, MD,[1] Masako Iwanaga, MD, PhD,[2] Daisuke Okamura, MD,[1] Maho Ishikawa, MD,[1] Tomoya Maeda, MD,[1] Tomoko Hata, MD,[2] Nobutaka Kawai, MD,[1] Yasushi Miyazaki, MD,[2] Masami Bessho, MD,[1] and Masao Tomonaga, MD[3]

From the [1]Department of Hemato-Oncology, Saitama International Medical Center, Saitama Medical University, Saitama, Japan; [2]Department of Hematology, Molecular Medicine Unit, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; and [3]Department of Hematology, Japanese Red Cross Nagasaki Atomic Bomb Hospital, Nagasaki, Japan.

Key Words: Myelodysplastic syndromes; Cytopenia; Dysplastic features; WHO classification

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ABSTRACT

Objectives: *To analyze the correlation between dysplastic lineage and type of cytopenia in myelodysplastic syndromes.*

Methods: *We analyzed the correlation between dysplasia and cell count using the data set of our previous morphologic study.*

Results: *There were no correlations between dysgranulopoiesis of 10% or more and absolute neutrophil count (ANC). Similarly, hyposegmented mature neutrophils (Pelger) of 10% or more were not related to ANC. Interestingly, the platelet count of patients with dysmegakaryopoiesis (dys Mgk) was higher than that of patients without dys Mgk (dys Mgk ≥10% vs <10%,* P = *.08; dys Mgk ≥40% vs <40%,* P = *.02; micromegakaryocytes ≥10% vs <10%,* P = *.004).*

Conclusions: *Since low cell counts did not correlate with the presence of dysplastic features, we suggest that dysplastic features do not directly relate to apoptosis.*

Upon completion of this activity you will be able to:
- list the dysplasia(s) in bone marrow for diagnosis of myelodysplastic syndromes (MDS).
- describe the mechanism of cytopenia(s) in MDS patients.
- define MDS subtypes according to 2008 World Health Organization criteria.

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Questions appear on p 277. Exam is located at www.ascp.org/ajcpcme.

Myelodysplastic syndromes (MDS) are very heterogeneous in terms of their cytomorphology, clinical features, and survival.[1] In 1982, the French-American-British (FAB) classification for the diagnosis of MDS was proposed,[2] and the current World Health Organization (WHO) classification[3] was proposed in 2008. The chapter on refractory cytopenia with unilineage dysplasia of the WHO classification described that "the type of cytopenia in the majority of cases will correspond to the type of dysplasia, e.g. anemia and erythroid dysplasia."[4] Although many groups have reported the cytomorphologic findings of MDS, to our knowledge a detailed analysis of the relationship between dysplastic lineage and the type of cytopenia has not been completely studied. Therefore, the correlation between dysplastic lineage and the type of cytopenia is unclear. Patients with MDS who do not exhibit a correlation between dysplastic lineage and the type of

cytopenia certainly exist. For example, MDS associated with isolated del(5q) (5q– syndrome) shows remarkable dysplastic features of megakaryocytic lineage in bone marrow (BM). However, the platelet counts of patients with 5q– syndrome do not usually decrease.[3] Previously, we reported a detailed cytomorphologic analysis of refractory anemia according to the FAB classification (FAB-RA).[5] Using this data set, we analyzed the relationship between dysplastic lineage and cell count in the present study.

Table 1
Patient Characteristics

Characteristic	Value
Age, median (range), y	57 (15-88)
Male sex, No. (%)	53 (53)
Hb, median (range), g/dL	8.3 (2.9-14.3)
ANC, median (range), $\times 10^9$/L	1.397 (0.260-6.201)
PLT, median (range), $\times 10^9$/L	35 (4-760)
Chromosome (IPSS), No. (%)	
Good	76 (76)
Intermediate	15 (15)
Poor	9 (9)

ANC, absolute neutrophil count; Hb, hemoglobin; IPSS, International Prognostic Scoring System; PLT, platelet count.

Materials and Methods

Patients

The data set of Japanese patients from our previous study[5] was used for this study. Patients included those with primary MDS excluding refractory anemia with ringed sideroblasts, refractory cytopenia with multilineage dysplasia and ringed sideroblasts, refractory anemia with excess of blasts (RAEB), or 5q– syndrome according to WHO classification (version 3).[6] Therefore, all patients in this study had FAB-RA except those with 5q– syndrome. Patients were diagnosed at the Saitama Medical University Hospital, Nagasaki University Hospital, or affiliated hospitals between April 1976 and January 2002. BM cellularity and fibrosis were evaluated by BM trephine biopsy and/or clot section. Patients with BM fibrosis were excluded, because the accuracy of morphologic evaluations might be not reliable owing to the few BM cells of the films. Disorders other than MDS (eg, aplastic anemia, paroxysmal nocturnal hemoglobinuria, megaloblastic anemia, autoimmune hemolytic anemia, anemia of chronic disorders, large granular lymphocytic leukemia, hairy cell leukemia, chronic liver disorders, and hypersplenism) were excluded. Patients who had previous therapy (antineoplastic drugs and/or ionizing radiation) or other prior hematologic disease also were excluded from the study. This study was approved by the Institutional Review Board of Saitama International Medical Center, Saitama Medical University. Retrospective analysis was performed in 100 Japanese patients. Age, sex, and cytogenetic findings of patients at diagnosis are summarized in **Table 1**.

Cytomorphologic Study

Microscopic examinations were performed using standard methods (BM Wright-Giemsa [WG] or May-Giemsa [MG], Prussian blue and periodic acid–Schiff [PAS] stained films, and peripheral blood [PB] WG- or MG-stained films).

In the present study, we analyzed the correlation between dysplasia and cell count using the data set of our previous morphologic study, in which we performed a detailed cytomorphologic analysis. We limited dysplasias to only dysplasias described in the WHO classification (version 3)[6] as follows. Dysplasias of the nucleus in erythroid lineage cells were defined as having budding, bridging, internuclear, karyorrhexis, multinuclearity, or megaloblastoid changes. Dysplasias of the cytoplasm in erythroid lineage cells were defined as having ring sideroblasts, vacuolization, or PAS positivity (diffuse or granular). With regard to granulocytes, dysplasias were defined as having the following characteristics: small size, nuclear hyposegmented mature neutrophils (Pelger), hypersegmentation, hypogranularity, or pseudo–Chédiak-Higashi granules. Dysplasias of megakaryocytes were defined as having micromegakaryocytes (mMgk), nonlobulated nuclei, or multiple widely separated nuclei. A minimum of 25 megakaryocytes, 200 erythroblasts, and 200 neutrophils in BM were examined in each patient. The cutoff levels for dyserythropoiesis (dys E) and dysgranulopoiesis (dys G) were defined as 10% according to the WHO classification.[6] Dysmegakaryopoiesis (dys Mgk) was evaluated with 2 cutoff levels: 10% according to the WHO classification or 40% according to data previously reported from the German group.[7,8] Since the accuracy of the quantitative evaluation of dysmegakaryopoiesis might be not reliable when there are few megakaryocytes to examine, we excluded patients who did not have at least 25 examined megakaryocytes from the morphologic evaluation of the megakaryocytic lineage. In our previous study, we reported that 2 distinct dysplastic changes, Pelger and mMgk **Image 1**, had negative prognostic impacts. Therefore, Pelger and mMgk were also evaluated. We defined hyposegmented mature neutrophils with strikingly clumpy chromatin as "Pelger" and mononucleated or binucleated megakaryocytes with a size equal to or smaller than promyelocytes as "mMgk." Positivity for Pelger (Pelger+) was defined as the presence of 10% or more Pelger among 200 mature neutrophils. Positivity for mMgk (mMgk+) was defined as the presence of 10% or more mMgk among 25 or more megakaryocytes. Patients with decreased megakaryocytes were assessed as being negative for mMgk (mMgk–).

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Definition of Cytopenias

To analyze the relationship between dysplasia and cytopenia, we compared hemoglobin (Hb) concentrations, absolute neutrophil counts (ANCs), platelet counts, and types of cytopenia. The definitions of cytopenias were as follows: Hb concentration less than 10 g/dL, ANC less than 1.8×10^9/L, and platelet count less than 100×10^9/L.[3]

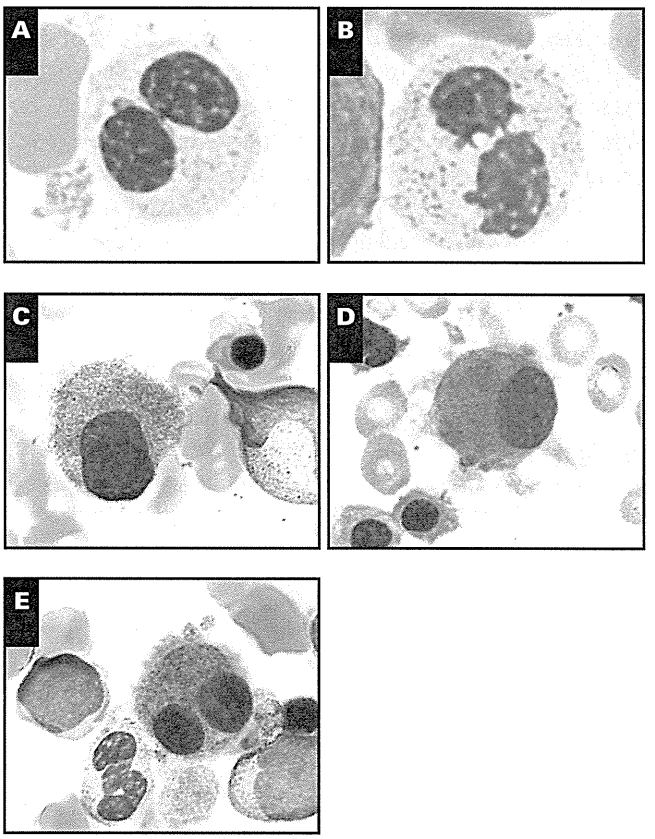
Statistical Analysis

Continuous data were compared using the nonparametric Mann-Whitney test, and proportions were compared using the χ^2 test. A 2-sided *P* value of less than .05 was considered statistically significant.

Results

Cytomorphologic Study

In our previous data set, we evaluated suitable BM preparations for the detailed assessments of myelodysplasia.[5] Results of morphologic analysis are shown in ▮Table 2▮ and ▮Table 3▮. Some BM preparations could not be examined in detail. In particular, observing the granules of neutrophils was difficult due to the poor staining condition of the films. Four cases could not be evaluated for the frequency of dys G, and 1 case could not be evaluated for the frequency of Pelger. Nineteen patients showed decreased megakaryocytes. Therefore,



▮Image 1▮ **A** and **B**, Nuclear hyposegmented mature neutrophils (Pelger) (May-Giemsa). **C-E**, Micromegakaryocytes (May-Giemsa).

▮Table 2▮
Relationship Between Dysgranulopoiesis and Absolute Neutrophil Count

Characteristic	No. of Patients	ANC, Median (Range), $\times 10^9$/L	*P* Value	No. (%) of Patients With Neutropenia[a]	*P* Value
dys G ≥10%	17	1.394 (0.492-6.201)	.83	13 (76.5)	.22
dys G <10%	79	1.397 (0.260-4.708)		48 (60.8)	
Pelger ≥10%	12	1.364 (0.492-6.201)	.86	9 (75.0)	.42
Pelger <10%	87	1.394 (0.260-4.708)		55 (63.2)	

ANC, absolute neutrophil count; dys G, dysgranulopoiesis; Pelger, nuclear hyposegmented mature neutrophils.
[a] Definition of neutropenia is ANC $<1.8 \times 10^9$/L.

▮Table 3▮
Relationship Between Dysmegakaryopoiesis and Platelet Count

Characteristic	No. of Patients	Platelets, Median (Range), $\times 10^9$/L	*P* Value	No. (%) of Patients With Thrombocytopenia[a]	*P* Value
dys Mgk ≥10%	69	48 (8-760)	.08	51 (73.9)	.18
dys Mgk <10%	12	26 (5-313)		11 (91.7)	
dys Mgk ≥40%	38	70 (15-760)	.02	26 (68.4)	.10
dys Mgk <40%	43	36 (5-343)		36 (83.7)	
mMgk ≥10%	12	99 (29-760)	.004	7 (58.3)	.03
mMgk <10%	88	33 (4-390)		7 (84.1)	

dys Mgk, dysmegakaryopoiesis; mMgk, micromegakaryocytes.
[a] Definition of thrombocytopenia is platelet count $<100 \times 10^9$/L.

these cases could not be evaluated for the frequency of dys Mgk. All patients showed dys E of 10% or more. No patients with unilineage dysplasia had dys G or dys Mgk of 10% or more. Patients with unilineage dysplasia had an erythroid lineage. Seventeen (18%) and 12 (12%) patients had dys G of 10% or more and were Pelger+, respectively. Sixty-nine (85%) and 38 (47%) patients had dys Mgk of 10% or more and 40% or more, respectively. Twelve (12%) patients were mMgk+. All patients with mMgk+ showed dys Mgk of 40% or more.

Table 4
Dysplastic Lineage(s) in Patients With Unilineage Cytopenia[a]

Case No.	dys E ≥10%	dys G ≥10%	dys Mgk ≥10%
Patients with only anemia			
103	+	−	+
131	+	+	−
136	+	−	+
257	+	−	+
Patients with only neutropenia			
135	+	+	+
230	+	+	+
245	+	−	+
286	+	−	+
Patients with only thrombocytopenia			
129	+	−	−
154	+	−	+
233	+	−	+
248	+	−	Unknown[b]
252	+	−	−
269	+	−	+
270	+	+	+
271	+	−	+
273	+	−	+
278	+	−	+
290	+	−	+

dys E, dyserythropoiesis; dys G, dysgranulopoiesis; dys Mgk, dysmegakaryopoiesis.
[a] Definitions of cytopenias are hemoglobin concentration <10 g/dL, absolute neutrophil count <1.8 × 10^9/L, and platelet count <100 × 10^9/L.
[b] Could not be evaluated for the frequency of dys Mgk due to decreases in megakaryocytes.

Table 5
Relationship Between Dysplastic Lineage and the Type of Cytopenia in Patients With Unilineage Dysplasia[a]

Case No.	Dysplastic Lineage	Anemia	Neutropenia	Thrombocytopenia
109	Erythroid	+	+	+
120	Erythroid	−	+	+
125	Erythroid	+	+	+
127	Erythroid	−	+	+
129	Erythroid	−	−	+
134	Erythroid	+	−	+
140	Erythroid	+	−	+
202	Erythroid	+	+	+
204	Erythroid	+	+	+
236	Erythroid	+	+	+
252	Erythroid	−	−	+

[a] Definitions of cytopenias are hemoglobin concentration <10g/dL, absolute neutrophil count <1.8 × 10^9/L, and platelet count <100 × 10^9/L.

Relationship Between Dysplasia and Cell Count

Results of the relationship between dysplasia and cell count are shown in Tables 2 and 3. There was no relationship between the presence of dys G of 10% or more and ANC. Similarly, Pelger+ was not related to ANC. Interestingly, platelet counts of patients with dys Mgk of 10% or more tended to be higher than those of patients without dys Mgk of 10% or more (P = .08). Moreover, in patients with dys Mgk of 40% or more, this difference in platelet counts was significant (dys Mgk ≥40% vs <40%, P = .02). In particular, in patients with mMgk+, the sign became even clearer (P = .004). In patients with dys Mgk of 40% or more, the platelet count of those with mMgk+ was not different from those without mMgk+ (P = .47). Of the 19 patients who showed unilineage cytopenia, only 2 had unilineage dysplasia. However, dysplastic lineages were different from the lineages of cytopenia **Table 4**. Of the 11 patients who showed unilineage dysplasia, only 2 had unilineage cytopenia. However, lineages of cytopenia were different from dysplastic lineages **Table 5**.

Discussion

Generally, it seems that cytopenias correspond to dysplastic lineage, but this has not been studied completely. Increases in blasts in BM may reduce blood cell counts due to hematopoietic injury. Patients with 5q− syndrome show anemia due to erythroid hypoplasia.[9,10] Increases in blasts or the existence of del(5q) may influence the decrease in cell count. To examine the correct relationship between dysplastic lineage and cell count, we excluded patients with RAEB or 5q− syndrome. Our previous data set used for the present study also does not include these cases. Therefore, it seems that this data set is suitable for the purpose of the present study.

Recently, it was reported that there was no clear correlation between the presence of any distinct dysplastic sign and cell counts in patients with MDS.[11] In the present study, we could not find any correlation between the presence of dys G of 10% or more and ANC. Low ANC cannot be directly explained by the presence of dys G. This result suggests that dysplastic features in granulocytic lineage may be unrelated to ineffective hematopoiesis explained by apoptosis. Interestingly, the platelet count of patients with dys Mgk was higher than that of patients without dys Mgk (dys Mgk ≥10% vs <10%, P = .08; dys Mgk ≥40% vs <40%, P = .02; mMgk+ vs mMgk−, P = .004). Although patients with 5q− syndrome show remarkable dysplastic features of megakaryocytic lineage in BM, their platelet counts are usually normal or increased. Thrombocytopenia is uncommon.[3,9,10] In the present study, a correlation between dys Mgk and platelet counts was similar to the characteristics of 5q− syndrome. These findings suggest that dys Mgk is not related to a direct sign of apoptosis, at least in the megakaryocytic lineage. In addition, we analyzed

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the correlation between the frequency of dys Mgk and the number of megakaryocytes. Evaluation of the megakaryocyte count was performed using specimens of the BM trephine biopsy and/or clot section. Of the 38 patients with dys Mgk of 40% or more, 21 (55%), 10 (26%), and 7 (17%) had increased, normal, and decreased megakaryocyte counts, respectively. In patients with dys Mgk of 40% or more, megakaryocyte counts tended to increase. In contrast, of the 43 patients without dys Mgk of 40% or more, 5 (12%), 17 (40%), and 21 (48%) had increased, normal, and decreased megakaryocyte counts, respectively. In patients without dys Mgk of 40% or more, megakaryocyte counts did not tend to increase. This finding was similar to a correlation between megakaryocyte count and mMgk. In 12 patients with mMgk+, 9 (75%), 3 (25%), and 0 had increased, normal, and decreased megakaryocyte counts, respectively. In patients with mMgk+, megakaryocyte counts tended to increase. In contrast, of the 88 patients without dys mMgk+, 17 (19%), 24 (27%), and 47 (53%) had increased, normal, and decreased megakaryocyte counts, respectively. In patients without mMgk+, megakaryocyte counts did not tend to increase. Increases in megakaryocyte counts may be a reason for the increase in platelet counts in patients with dys Mgk of 40% or more or mMgk+.

Only 6 patients had hypoplastic BM. There was no significant difference in Hb concentrations between hypoplastic BM and nonhypoplastic BM patients (P = .51). Similarly, there was no significant difference in ANC between the 2 groups (P = .44). Nineteen patients had decreased megakaryocytes. Interestingly, the platelet count of patients with decreased megakaryocytes (median, 18 × 10^9/L) was significantly lower than that of patients without decreased megakaryocytes (median, 48 × 10^9/L) (P < .001). This finding is similar to the mechanism of thrombocytopenia in patients with aplastic anemia. It seems that the presence of decreased megakaryocytes may be associated with the presence of thrombocytopenia even in patients with MDS.

We could not find a correlation between dysplastic lineage and low cell counts. Low ANC cannot be explained by the presence of dys G. The platelet count of patients with dys Mgk was higher than that of patients without dys Mgk. Therefore, we suggest that dysplastic features do not directly relate to apoptosis in MDS patients with FAB-RA except 5q– syndrome. To clarify the conclusion of our study, quantitative analysis of apoptosis by immunohistochemistry should be performed in the future studies.

Address reprint requests to Dr Matsuda: Dept of Hematology, Saitama International Medical Center, Saitama Medical University, 1397-1 Yamane, Hidaka, Saitama, 350-1298, Japan; e-mail: amatsu@saitama-med.ac.jp.

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CD56 expression is an independent prognostic factor for relapse in acute myeloid leukemia with t(8;21)

Noriyoshi Iriyama[a,*], Yoshihiro Hatta[a], Jin Takeuchi[a], Yoshiaki Ogawa[b], Shigeki Ohtake[c], Toru Sakura[d], Kinuko Mitani[e], Fumihiro Ishida[f], Masatomo Takahashi[g], Tomoya Maeda[h], Tohru Izumi[i], Hisashi Sakamaki[j], Shuichi Miyawaki[k], Sumihisa Honda[l], Yasushi Miyazaki[m], Tomohiko Taki[n], Masafumi Taniwaki[o], Tomoki Naoe[p]

[a] *Department of Hematology and Rheumatology, Nihon University Itabashi Hospital, Tokyo, Japan*
[b] *Department of Hematology and Oncology, Tokai University School of Medicine, Kanagawa, Japan*
[c] *Department of Clinical Laboratory Science, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan*
[d] *Leukemia Research Center, Saiseikai Maebashi Hospital, Gunma, Japan*
[e] *Department of Hematology and Oncology, Dokkyo Medical University School of Medicine, Tochigi, Japan*
[f] *Division of Hematology, Department of Internal Medicine, Shinshu University School of Medicine, Nagano, Japan*
[g] *Division of Hematology and Oncology, Department of Internal Medicine, St. Marianna University School of Medicine, Kanagawa, Japan*
[h] *Department of Hematology, Saitama Medical University International Medical Center, Saitama, Japan*
[i] *Department of Hematology, Tochigi Cancer Center, Tochigi, Japan*
[j] *Department of Hematology, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan*
[k] *Department of Internal Medicine, Tokyo Metropolitan Ohtsuka Hospital, Tokyo, Japan*
[l] *Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan*
[m] *Department of Hematology, Atomic Bomb Disease and Hibakusha Medicine Unit, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan*
[n] *Department of Molecular Diagnostics and Therapeutics, Kyoto Prefectural University of Medicine Graduate School of Medical Science, Kyoto, Japan*
[o] *Department of Hematology and Oncology, Kyoto Prefectural University of Medicine Graduate School of Medical Science, Kyoto, Japan*
[p] *Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Aichi, Japan*

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ABSTRACT

We investigated the significance of surface antigen expression for prognosis by focusing on a specific subtype, AML with t(8;21). The investigation included 144 patients with AML with t(8;21) in the JALSG AML97 study. AML with t(8;21) expressed CD19 (36%), CD34 (96%), and CD56 (65%) more frequently than did other subtypes of AML. CD19 expression had a significant favorable effect on CR (95.7% vs. 83.8%; $P=0.049$). Univariate analysis showed that increased white blood cell (WBC) counts (WBC $\geq 20 \times 10^9$/L), CD19 negativity, and CD56 positivity were critical adverse factors for relapse after CR; multivariate analysis revealed that WBC count and CD56 expression were independent adverse risk factors (HR 2.18; $P=0.045$, HR 2.30; $P=0.011$, respectively). We concluded that CD56 expression has a possible role in risk stratification for patients with AML with t(8;21).

1. Introduction

In recent years, immunophenotyping of hematologic neoplasms has become standard practice in the determination of diagnosis and the definition of origin cell lineage. Patients with acute myeloid leukemia (AML) often demonstrate aberrant cellular antigen expression as well as chromosomal abnormalities. The clinical significance of surface antigen expression has been studied for more than 20 years, but with few consistent results [1]. It is thought that the heterogeneity of AML and/or the impact of subtypes and their chromosomal abnormalities on prognosis makes it difficult to conclusively interpret the significance of surface antigen expression. In addition, most previous studies included varying treatment protocols, were influenced by the effects of hematopoietic stem cell transplantation (HSCT) and were conducted in a retrospective fashion. One is more likely to find a significant relationship between surface antigens and prognosis by limiting examination to a particular subtype of AML. For example, the significance of CD56 expression as an adverse prognostic factor in acute promyelocytic leukemia (APL) treated with all-*trans* retinoic acid and anthracycline-based regimen is in little doubt [2].

* Corresponding author at: Department of Hematology and Rheumatology, Nihon University Itabashi Hospital, 30-1 Oyaguchi Kamicho, Itabashi-ku, Tokyo 173-8610, Japan. Tel.: +81 3 39728111.
E-mail address: iriyama.noriyoshi@nihon-u.ac.jp (N. Iriyama).

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AML with t(8;21) is a specific subtype, usually classified as M2 according to the French–American–British (FAB) classification system. This subtype is considered to be clinically favorable, and more than half of patients are cured with chemotherapy [3–7]. Although the prognosis of acute myeloid leukemia (AML) with t(8;21) is better than in other types of AML, patient outcome is not always satisfactory.

Interestingly, AML with t(8;21) frequently expresses the immature B-cell linage marker CD19 and the primitive marker CD34 together [8–10]. In addition, some reports suggested that aberrant CD56 expression was a risk factor in AML with t(8;21) [11,12]. However, the number of cases in these studies was small and the chemotherapy regimens that were used varied. AML with t(8;21) occurs comparatively frequently in the Japanese population [13]. In the present study, we used the data from Japan Adult Leukemia Study Group (JALSG) AML 97 to investigate the clinical significance for prognosis of surface antigen expression in patients with AML with t(8;21). Our investigation included 144 patients with AML with t(8;21), the largest number of cases to be studied to date.

2. Patients and methods

2.1. Patients

We conducted a retrospective review of patient data from the multicenter JALSG AML97 study; detailed information regarding this study and its results has been previously reported [14]. Briefly, patients aged 15–64 years with de novo AML, excluding those with APL, were consecutively registered from 103 participating institutions between December 1997 and July 2001. AML was diagnosed according to the FAB classification system at each institution. Patients with prediagnosed myelodysplastic syndrome and those who had been exposed to chemotherapy were excluded from this study. Of 809 patients with newly diagnosed AML, 789 were eligible for the study. Informed consent was obtained from all patients before enrollment. In accordance with the Declaration of Helsinki, the study protocol was approved by the research ethics board of each participating hospital.

2.2. Cytogenetic studies

Results of the cytogenetic studies performed at each institution were reported to the JALSG Statistical Center before treatment. Twenty metaphases were routinely counted and analyzed in each patient according to International System for Human Cytogenetic Nomenclature (ISCN) recommendations.

2.3. Flow cytometry

Immunophenotyping was performed at each institution, primarily on freshly collected bone marrow or peripheral blood samples at the time of diagnosis. Leukemic cell analysis was performed at local or reference laboratories by standard immunofluorescence methods using monoclonal antibodies directed against CD2, CD3, CD4, CD5, CD7, CD8, CD11b, CD13, CD15, CD19, CD33, CD34, CD41a, CD56, and HLA-DR surface antigens. Samples were considered positive if at least 20% of blasts expressed the antigen.

2.4. Treatment and outcome

The detailed treatment protocol has been previously reported [14]. Patients who obtained complete remission (CR) within 2 courses of induction therapy were randomized into groups that received either 4 courses of standard-dose consolidation therapy without maintenance (Arm A) or 3 courses of standard-dose consolidation and 6 courses of maintenance therapy (Arm B). No statistical difference was observed between the arms in either the 5-year overall survival (OS) rate or the 5-year relapse-free survival rate in a previous study [14].

2.5. Statistical analysis

OS for all patients was defined as the interval from the date of diagnosis to the date of death. Cumulative incidence of relapse (CIR) was defined as the interval from the date of CR to the date of the first recurrence. Any patients who underwent HSCT were censored from the analysis of relapse on the date of this treatment.

The Kaplan-Meier method was used to estimate OS and CIR. The log-rank test was used to compare OS or CIR between 2 groups. Factors that could potentially affect clinical outcome, including age, sex, WBC count, performance status at diagnosis, allocation to consolidative treatment, and surface antigen expression, were analyzed by the multivariate Cox proportional hazard regression model. Fisher's exact test and Student's *t*-test were applied to compare factor differences between 2 groups. Statistical analysis was performed with JMP software version 8.0.1 (SAS Institute Inc., Cray, NC, USA).

3. Results

3.1. Distribution of surface antigen expression

There were 789 patients eligible for the study. CD2, CD4, CD7, CD11b, CD13, CD15, CD19, CD33, CD34, CD56, and HLA-DR were examined in 701, 648, 707, 517, 722, 450, 713, 724, 715, 652, and 710 patients, respectively. Of the 781 patients remaining when 8 with undetectable karyotypes were excluded, 144 were diagnosed with t(8;21). CD2, CD4, CD7, CD11b, CD13, CD15, CD19, CD33, CD34, CD56, and HLA-DR were examined in 124, 115, 127, 95, 129, 80, 127,

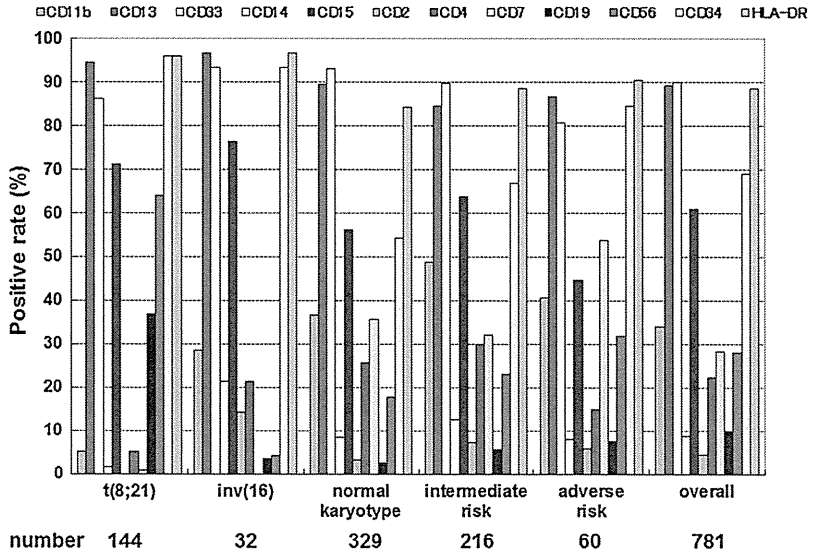

Fig. 1. Distribution of surface antigen expression. AML with t(8;21) is compared with other karyotypes of AML. Patients were classified into t(8;21), inv(16), normal karyotype, intermediate-risk (excluding normal karyotype), or adverse-risk group. Positive rates of the surface antigens CD11b, CD13, CD33, CD14, CD15, CD2, CD4, CD7, CD19, CD56, CD34, and HLA-DR are indicated. The overall category represents the 781 patients whose karyotypes were detectable.

130, 127, 117, and 126 patients, respectively. Of the 144 AML with t(8;21) patients, 7 had upfront HSCT after CR.

We investigated the distribution of surface antigen expression based on cytogenetic risk classification according to Medical Research Council [5]; these results are shown in Fig. 1. AML with t(8;21) commonly expressed CD13, CD33, CD34, and HLA-DR. In contrast, the expression of CD11b was uncommon. CD19 was observed frequently in AML with t(8;21); CD15 and CD56 were also frequently observed in the t(8;21) group. We then analyzed the relationship between surface antigen expression and clinical outcome, focusing on CD15, CD19, and CD56.

3.2. CR rate

CR rates are shown in Table 1. The overall CR rate was 88.9%, higher than the overall CR rate of 78.7% in the entire AML97 study population [14]. CD19 positivity was a common factor in patients who obtained CR. CD15 and CD56 were not significantly associated with the CR rate.

Table 1
Correlation between complete remission (CR) rate and surface antigen expression.

Factors	CR rate (%) positive group	CR rate (%) negative group	*P* value
CD15	50/57(87.7%)	21/23(91.3%)	1.000
CD19	45/47(95.7%)	67/80(83.8%)	0.049
CD56	65/75(86.7%)	37/42(88.1%)	1.000

3.3. Relapse risk

In total, 128 patients achieved CR and were randomized to the 2 consolidative regimens [14]. Univariate analysis showed significantly unfavorable outcomes in patients with increased white blood cell (WBC) counts (WBC $\geq 20 \times 10^9$/L) at diagnosis and those who were CD56 positive and CD19 negative (Table 2, Fig. 2).

Other factors such as age ≥50 years, female sex, poor performance status at diagnosis, and allocation to consolidative treatment in Arm B had a negative impact on prognosis, being associated with relapse. Importantly, however, multivariate analysis showed WBC count and the presence of CD56 expression to be

Table 2
Analysis of prognostic factors for relapse.

Factors	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age ≥50	1.34 (0.79–2.23)	0.271		
Female sex	1.11 (0.65–1.84)	0.686		
WBC $\geq 20 \times 10^9$/L	1.89 (1.01–3.34)	0.048	2.18 (1.02–4.25)	0.045
Performance status ≥2	1.32 (0.61–2.55)	0.453		
Allocation (B arm)	1.40 (0.85–2.32)	0.192		
CD15 positive	1.66 (0.78–3.60)	0.203		
CD19 positive	0.56 (0.30–0.99)	0.046	0.64 (0.33–1.16)	0.142
CD56 positive	2.20 (1.18–4.41)	0.013	2.30 (1.20–4.77)	0.011

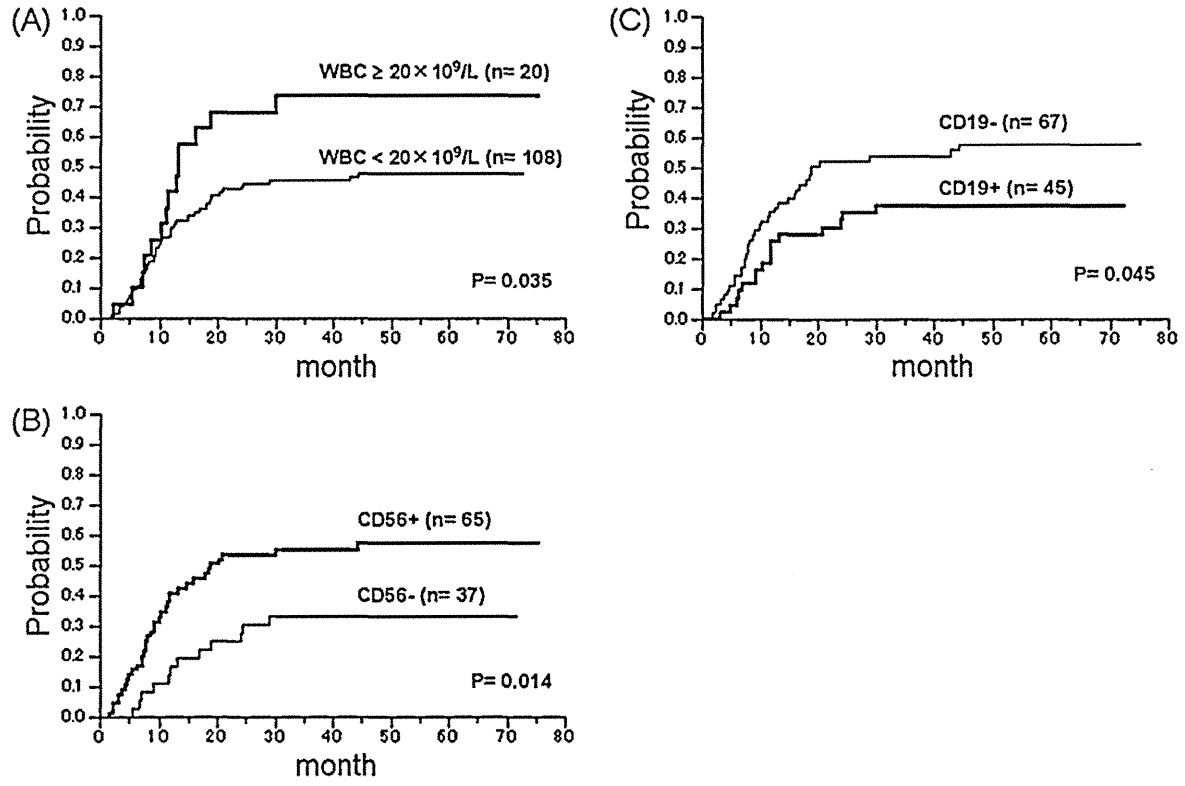

Fig. 2. Kaplan-Meier curves indicating cumulative incidence of relapse according to white blood cell count (WBC) (A), CD56 expression (B), and CD19 expression (C). Log-rank testing showed significant differences in the rate of relapse.

Table 3
Analysis of prognostic factors for overall survival.

Factors	Univariate analysis	
	HR (95% CI)	*P* value
Age ≥50	1.36 (0.78–2.32)	0.270
Female sex	0.99 (0.56–1.69)	0.966
WBC ≥20×10^9/L	1.43 (0.75–2.54)	0.261
Performance status ≥2	1.15 (0.53–2.23)	0.698
CD15 positive	1.53 (0.70–3.85)	0.299
CD19 positive	0.68 (0.36–1.23)	0.209
CD56 positive	1.81 (0.96–3.65)	0.065

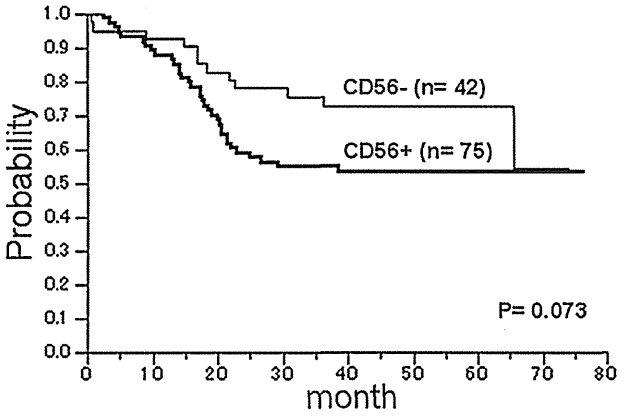

Fig. 3. Kaplan-Meier curves indicating overall survival (OS) according to CD56 expression. CD56 expression did not affect OS.

independent prognostic factors for relapse. Other factors did not affect the relapse rate.

3.4. OS

To investigate whether the expression of CD15, CD19, and CD56 affect OS, we used univariate analysis. Unlike its effect on relapse risk, CD56 did not affect OS ($P = 0.073$, Fig. 3) Likewise, CD 15 and CD19 did not have an impact on OS ($P = 0.314$, 0.215, respectively; Table 3).

3.5. Correlation between CD56 expression and other factors

As CD56 expression was detected as an independent prognostic factor for relapse, we next analyzed the correlation between CD56 expression and other factors; these results are shown in Table 4. CD56 positive or negative status was not associated with patient age, sex, WBC count, presence or absence of Auer rods, performance status, CD15 expression, or the presence of additional cytogenetic abnormalities including -*X*/-*Y* and del(9q). In contrast, CD19-positive patients were more commonly observed in the CD56 negative group than in the CD56 positive group (51% vs. 31%, respectively).

4. Discussion

We were able to demonstrate the significance of CD56 expression as a predictor of relapse in patients with AML with t(8;21). Our patient series is larger than that of previous studies, and we were able to analyze data from a well-designed prospective study.

We observed that patients with AML with t(8;21) frequently expressed the surface antigens CD34, CD15, CD19, HLA-DR, and CD56; expression of CD11b was rare compared with other AML subtypes. As CD11b is known to be associated with poor prognosis in patients with AML [1], its rare appearance in AML with t(8;21) is in accordance with the good prognosis of patients with this subtype. It is also known that the expression of CD7 and CD34 is associated with poor clinical outcome in AML [1].

We found that the expression of CD7, CD56, and CD34 were frequently observed in adverse karyotype group, suggesting that the impact on clinical outcome of cytogenetics overlaps with that of surface antigen expression: the presence or absence of these antigens seems to have clinical significance. However, the fact that CD34 and CD56 are also frequently observed in t(8;21) AML, suggests that surface antigens are not the only factors that determine outcome in patients with AML. These results led us to investigate the association between surface antigens and prognosis in a certain subtype of AML.

In our study limited to AML with t(8;21), univariate analysis showed increased WBC counts (WBC ≥ 20×10^9/L), CD56 positivity, and CD19 negativity were unfavorable factors for relapse. It has been previously shown that WBC ≥ 20×10^9/L and female sex are predictive factors for relapse [1,14,15]. Our study findings are in agreement with the findings of these reports in that WBC ≥ 20×10^9/L was a prognostic factor. However, patient gender did not affect the risk of relapse in our patients. A previous investigation by Baer et al. [12] showed similar results to our study in that patient characteristics such as WBC count, age, and sex did not correlate with CD56 expression, and that CD56 expression affected relapse, although the number of patients in the study was small. Our larger study demonstrated that CD56 is an independent prognostic factor for relapse in AML with t(8;21). We found, however, that the impact of CD56 expression on OS was not significant. Our study limitations include the fact that 3 patients who failed to achieve CR, and 22 patients who relapsed after consolidative therapy were treated with HSCT, with or without salvage chemotherapy. It is also worth noting that salvage chemotherapy and/or HSCT after treatment failure or relapse may have influenced our results.

It has been shown that CD56 expression is significantly associated with P-glycoprotein (PGP) overexpression, a poor prognostic factor, as well as a reduced probability of achieving CR in AML

Table 4
Correlations in CD56 positive and negative populations. Ninety-seven cases reviewed centrally were presented for the analysis of additional cytogenetic abnormalities including -*X*/-*Y* and del(9q).

Factors	CD56 negative ($n = 42$)	CD56 positive ($n = 75$)	*P* value
Age (year), median (range)	41 (15–58)	43 (16–64)	0.271
Sex (male:female)	27:15	51:24	0.688
WBC ($\times 10^9$/L), median (range)	7.3 (2.7–65.8)	9.7 (1.7–119.5)	0.434
LDH (U/L), median (range)	736 (178–3575)	841 (164–3151)	0.704
Auer rod positive/negative	31/11	61/14	0.356
CD15 positive/negative	21/7	33/14	0.792
CD19 positive/negative	21/20	23/52	0.045
Performance status ≥2/0–1	9/33	9/66	0.191
-*X* or -*Y* positive/negative	18/16	36/27	0.831
del(9q) positive/negative	5/29	5/58	0.313

patients [16,17]. Hence, we are interested in whether high-dose Ara-c (HDAC) regimens can overcome poor prognostic factors such as CD56 positivity or PGP overexpression in patients with AML with t(8;21). Consolidative regimens with HDAC are believed to be more effective than conventional anthracycline-based regimens in patients with AML [18], so by extension HDAC is logically considered superior to anthracycline-based regimens against AML cells with overexpression of PGP. Since an HDAC regimen was not used in the study protocol, our study may show a worse prognosis for CD56-positive AML with t(8;21) than it otherwise might have. In addition, patients' PGP expression was not examined in this study. After the conclusion of AML97, JALSG conducted AML201 in which patients were randomized to HDAC- or anthracycline-based consolidation therapies as well as IDR + Ara-C and daunorubicin + Ara-C induction therapies [19,20]. This study did not demonstrate apparent superiority of the HDAC treatment [19]; we hypothesize however that HDAC treatment may be able to overcome the prognostic impact of CD56 expression in an unfavorable population.

In our study CD19, a major B-cell marker, was frequently observed in AML with t(8;21); this finding is in agreement with a previous report [8]. CD19 expression is significantly correlated with improved prognosis in the study population, probably because the CD56-negative population frequently demonstrates CD19 positivity.

Since the relationship between surface antigen expression and prognosis was made clear in this population, we are now interested in examining the association between surface antigen expression, the *KIT* mutation, and patient prognosis. Previous reports have shown that a presence of *KIT* mutation affects the prognosis in patients with AML with t(8;21) [21,22]. Furthermore, De et al. [23] suggested that a presence of *KIT* mutation in AML with t(8;21) were associated with CD19 negativity and CD56 positivity, but the number of cases in the study was small. JALSG is now investigating whether the presence or absence of the *KIT* mutation affects patient prognosis in the CBF-AML209-KIT study [24]. The relationships among surface antigen expression, molecular evaluation, and clinical features in AML with t(8;21) will be elucidated in future investigations.

Immunophenotypic analyses were not centrally performed in this study, preventing a systematic standardization of flow cytometry. Differences in reagents, gating and staining techniques, and thresholds for positivity may have caused discrepancies between centers. A further limitation of our study is possible selection bias, because not all centers performed cytometric analysis of all AML97 study antigens at the time of patient entry. This led to a reduction in sample size, as CD56 was not measured in 19% of patients with AML with t(8;21).

In conclusion, we have determined that CD56 expression has a possible role in risk stratification for patients with AML with t(8;21). CD 19 negativity, although predictive of relapse in univariate analysis, was not significant on multivariate analysis.

Conflicts of interest statement

There are no relevant conflicts of interest to disclose.

Acknowledgements

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Contributions. N.I., Y.H., S.H., and J.T.: analyzed results and presented; J.T., Y.O., S.O., T.S., K.M., F.I., M.(Masatomo)T., T.M., T.I., H.S., and S.M.: accumulated clinical data; H.S., S.M., Y.M., T.T., M.(Masafumi)T., and T.N.: assisted interpretation of the result; N.I., Y.H., J.T., H.S., and T.N.: designed the research; N.I., Y.H., and J.T.: wrote the manuscript; T.N.: directed and oversight the project.

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Original Article

The Demarcation Between Younger and Older Acute Myeloid Leukemia Patients

A Pooled Analysis of 3 Prospective Studies

Masamitsu Yanada, MD[1]; Shigeki Ohtake, MD[2]; Shuichi Miyawaki, MD[3]; Hisashi Sakamaki, MD[4]; Toru Sakura, MD[5]; Tomoya Maeda, MD[6]; Koichi Miyamura, MD[7]; Norio Asou, MD[8]; Iekuni Oh, MD[9]; Junichi Miyatake, MD[10]; Hiroyuki Kanbayashi, MD[11]; Jin Takeuchi, MD[12]; Masatomo Takahashi, MD[13]; Nobuaki Dobashi, MD[14]; Hitoshi Kiyoi, MD[15]; Yasushi Miyazaki, MD[16]; Nobuhiko Emi, MD[1]; Yukio Kobayashi, MD[17]; Ryuzo Ohno, MD[18]; and Tomoki Naoe, MD[15]; for the Japan Adult Leukemia Study Group

BACKGROUND: Contemporary treatment protocols for adult acute myeloid leukemia (AML) are age-specific, and older patients are generally treated less intensively than younger patients. However, it remains uncertain whether older but fit patients with AML really need to have their treatment attenuated. **METHODS:** To evaluate the contribution of age to outcome for patients with AML receiving intensive chemotherapy, data were analyzed for 2276 patients aged less than 65 years who were treated uniformly, regardless of age, in 3 consecutive prospective studies conducted by the Japan Adult Leukemia Study Group. **RESULTS:** A substantial drop in overall survival (OS) between patients aged 40 to 49 years and 50 to 64 years led to a focus on 2 comparisons: 1) age <50 versus ≥ 50 years; and 2) age 50 to 54 versus 55 to 59 versus 60 to 64 years. OS was significantly better for patients aged <50 years than that for those aged ≥ 50 years (49.6% and 37.0% at 5 years; $P<.001$); older patients were more susceptible to relapse, but not to early death or nonrelapse mortality. The significant differences in OS between these 2 age groups were equally seen for patients with favorable, intermediate, and adverse cytogenetics ($P<.001$ each). Outcomes for those aged 50 to 54, 55 to 59, and 60 to 64 years were similar, with 5-year OS rates of 38.2%, 35.1%, and 38.0%, respectively ($P=.934$), and no differences in early death or nonrelapse mortality were observed among these age groups. **CONCLUSIONS:** These findings justify the use of intensive chemotherapy without dose attenuation toward older but fit patients with AML, at least up to the age of 64 years. ***Cancer*** **2013;119:3326-33.** © *2013 American Cancer Society.*

KEYWORDS: acute myeloid leukemia; age; overall survival; early death; relapse; nonrelapse mortality.

INTRODUCTION

Age is among the most important prognostic factors in acute myeloid leukemia (AML).[1-5] Increasing age in AML is associated with a higher frequency of unfavorable biological characteristics such as adverse cytogenetics, preceding myelodysplastic syndrome (MDS), and expression of the multidrug resistance phenotype, all of which are involved in intrinsic resistance to chemotherapy.[6-9] In addition to the disease biology, patient-related factors such as poor general condition and significant comorbidities also contribute to inferior outcomes for older patients.[8,10,11] Because of such distinct biological and clinical features, contemporary treatment protocols for adult AML are age-specific and are typically divided into those for younger and older patients, with older patients treated less intensively than younger patients. For this purpose, age 55 or 60 years is generally used as the demarcation between these 2 groups[1,2]; however, this cutoff age is quite arbitrary, and it remains uncertain whether patients over such age limits really need to have their treatment attenuated.

For the recent prospective AML studies conducted by the Japan Adult Leukemia Study Group (JALSG), age less than 65 years was used as the eligibility criterion, with dose modifications not having been adopted according to age. This

Corresponding author: Masamitsu Yanada, MD, Department of Hematology, Fujita Health University School of Medicine, 1-98 Dengakugakubo, Kutsukake-cho, Toyoake, 470-1192, Japan; Fax: (011) +81-562-95-0016; myanada@fujita-hu.ac.jp

[1]Fujita Health University School of Medicine, Toyoake, Japan; [2]Kanazawa University Graduate School of Medical Science, Kanazawa, Japan; [3]Tokyo Metropolitan Ohtsuka Hospital, Tokyo, Japan; [4]Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan; [5]Saiseikai Maebashi Hospital, Maebashi, Japan; [6]Saitama Medical University International Medical Center, Saitama, Japan; [7]Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; [8]Kumamoto University School of Medicine, Kumamoto, Japan; [9]Jichi Medical University, Tochigi, Japan; [10]Kinki University Faculty of Medicine, Osaka, Japan; [11]Ohta Nishinouchi Hospital, Kohriyama, Japan; [12]Nihon University School of Medicine, Tokyo, Japan; [13]St. Marianna University School of Medicine, Kawasaki, Japan; [14]Jikei University School of Medicine, Tokyo, Japan; [15]Nagoya University Graduate School of Medicine, Nagoya, Japan; [16]Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; [17]National Cancer Center Hospital, Tokyo, Japan; [18]Aichi Cancer Center, Nagoya, Japan

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situation provides a welcome opportunity to evaluate the contribution of age to outcome for patients with AML treated with uniform intensive chemotherapy. For the study reported here, we integrated data for 2276 patients entered into 3 consecutive prospective studies between 1995 and 2005 for a comparison of patient characteristics and treatment outcomes among different age groups.

MATERIALS AND METHODS

Patients

All patients were subjects of one of the three phase 3 studies conducted by the JALSG, that is, the AML95 (from 1995-1997),[12] AML97 (from 1997-2001),[13,14] and AML201 (from 2001-2005) studies.[15,16] All of these studies adopted the same eligibility criteria: newly diagnosed AML (acute promyelocytic leukemia excluded), age 15 to 64, an Eastern Cooperative Oncology Group performance status 0 to 3, adequate functioning of the liver (serum bilirubin level < 2.0 mg/L), kidneys (serum creatinine level < 2.0 mg/dL), lungs ($PaO_2 \geq 60$ Torr or $SpO_2 \geq 93\%$), and heart (no significant abnormalities on electrocardiograms and echocardiograms). Patients with AML secondary to MDS or cytotoxic treatment were not eligible for enrollment. Written informed consent was obtained from all patients prior to registration. Each protocol was reviewed and approved by the institutional review boards of the participating centers, and was conducted in accordance with the Declaration of Helsinki.

Treatments

The treatment schedule for each study is described in detail elsewhere.[12-16] The AML95 study compared a fixed schedule (ie, "3+7") and an individualized schedule (up to "4+10" depending on the bone marrow findings on day 8) for induction therapy with idarubicin and cytarabine.[12] Postremission therapy consisted of 3 courses of consolidation therapy including behenoyl cytarabine and 12 months of maintenance therapy. The AML97 study adopted the 3+7 induction therapy with idarubicin and cytarabine for all patients.[13,14] After achieving complete remission (CR), patients were randomized to receive 3 or 4 consolidation courses that included standard-dose cytarabine, followed by 12 months of maintenance therapy only for the 3 courses. Those with a human leukocyte antigen (HLA)-identical sibling donor were assigned to allogeneic hematopoietic cell transplantation (HCT) if they were younger than 50 years and at intermediate or poor risk, as determined with a scoring system which took into account cytogenetics, white blood cell count, and other factors. The AML201 study compared idarubicin (12 mg/m^2 for 3 days) and daunorubicin (50 mg/m^2 for 5 days) both combined with cytarabine for induction therapy.[14,15] Patients in CR were randomly assigned to either 4 consolidation courses with standard-dose cytarabine or 3 courses with high-dose cytarabine. Allogeneic HCT was offered to patients aged 50 or younger if they presented with intermediate or adverse cytogenetics and had an HLA-identical sibling donor. In principle, doses were not modified according to age for any protocol. The single exception was for high-dose cytarabine in the AML201 study, in which reduction of the cytarabine dose from 2 g/m^2 to 1.5 g/m^2 was allowed for patients aged 60 years or older.

Definitions

Karyotypes were classified as favorable, intermediate, or adverse, in line with the revised UK Medical Research Council (MRC) criteria.[17] Monosomal karyotype was defined according to the criteria developed by Breems et al.[18]

CR was defined as the presence of all of the following: $< 5\%$ of blasts in bone marrow, no leukemic blasts in peripheral blood or extramedullary sites, and recovery of peripheral blood counts. Early death was defined as death from any cause occurring within 30 days after the start of induction therapy.[8] Overall survival (OS) was defined as the time from the start of treatment to death or last visit, and relapse-free survival as the time from CR to relapse, death or last visit. Patients undergoing allogeneic HCT were not censored at the time of transplantation unless indicated.

Statistical Analysis

Distributions of patient characteristics between and among groups were compared by using the chi-square test for categorical variables. Differences in continuous variables were compared by means of the Wilcoxon rank-sum test for distribution between 2 groups, and the Kruskal-Wallis test for distribution among 3 groups. The probabilities of OS and relapse-free survival were estimated by using the Kaplan-Meier method, with differences between groups qualified with the log-rank test. First, we examined OS by dividing patients into 4 age groups: 15-29, 30-39, 40-49, and 50-64 years. This provisional analysis disclosed a substantial drop in OS between patients aged 40-49 and 50-64 years (Fig. 1A). This finding led us to focus on 2 comparisons for subsequent analyses: 1) age < 50 versus ≥ 50 years; and 2) age 50 to 54 versus 55 to 59 versus 60 to 64 years. Relapse and nonrelapse mortality were considered as competing risk events for each other, and the probabilities of relapse