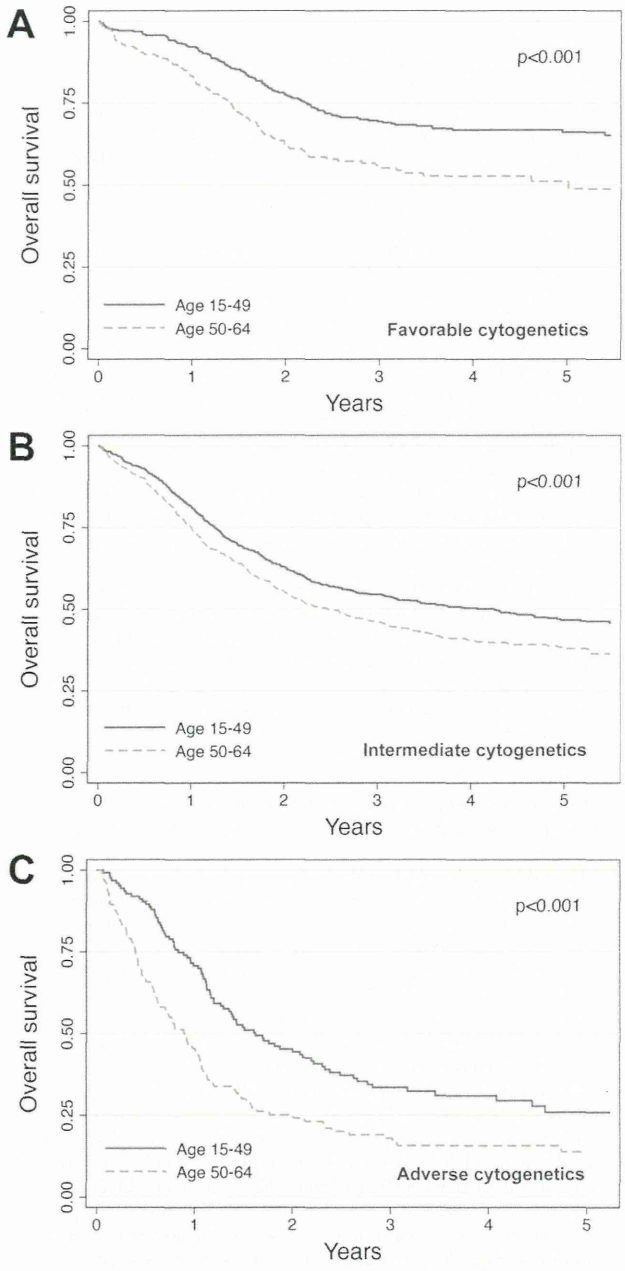

Figure 2. Overall survival for patients with each cytogenetic risk is shown by age group. Patients with (A) favorable (N = 515), (B) intermediate (N = 1461), and (C) adverse (N = 229) cytogenetics are shown separately.

TABLE 3. Multivariate Analysis of Risk Factors for Overall Survival

	HR	(95% CI)	*P*
Age			
<50	1.00		-
≥50	1.48	(1.32-1.66)	<.001
Sex			
Male	1.20	(1.07-1.36)	.002
Female	1.00		-
Performance status			
0-1	1.00		-
2-3	1.32	(1.12-1.56)	.001
White blood cell count			
Per 10 $\times 10^9$/L increase	1.02	(1.01-1.03)	<.001
Protocol			
AML95	1.25	(1.07-1.45)	.004
AML97	1.07	(0.94-1.22)	.318
AML201	1.00		-
Cytogenetics			
Favorable	0.65	(0.55-0.77)	<.001
Intermediate	1.00		-
Adverse	2.07	(1.75-2.45)	<.001
Unevaluable	1.41	(1.05-1.90)	.024

Abbreviations: CI, confidence interval; HR, hazard ratio.

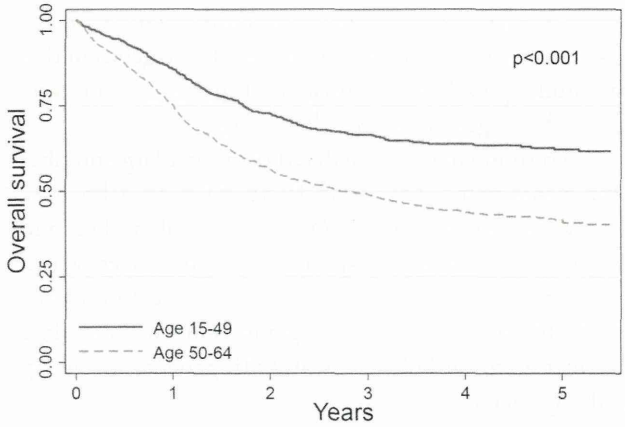

Figure 3. Overall survival is shown by age group with censoring of patients undergoing allogeneic hematopoietic cell transplantation. Patients aged 15 to 49 (N = 1339) and 50 to 64 years (N = 937) are compared, with those undergoing allogeneic hematopoietic cell transplantation censored at the time of transplantation.

When interpreting our data, we should bear in mind that our study cohort consisted exclusively of newly diagnosed AML patients under the age of 65 years who were entered into phase 3 studies. In addition, our cohort did not include patients with AML secondary to MDS or cytotoxic treatment. Secondary AML accounts for approximately 35% of the whole AML population,[26,27] and the frequency is even higher among older patients.[3,5] Our results therefore might not be applicable to the general AML population. This limitation may well be partly complemented by the findings of several previous studies. Buchner et al analyzed data of 2776 patients with de novo AML with no upper age enrolled onto 2 prospective studies by the German AML Cooperative Group.[9] In that study, OS for patients older than 60 years was only half that of younger patients, and this difference was attributable to less frequent CR and more frequent relapse in older patients. It seems likely that inclusion of elderly

patients might have contributed to a larger prognostic difference between younger and older patients. By using the combined data of 5 AML studies conducted by the Southwest Oncology Group, Appelbaum et al evaluated effect of age on outcomes.[8] Their study included not only patients with de novo AML but also those with secondary AML, with no upper age limit employed in trials for older patients. CR rates and OS were shown to worsen with advanced age, and this held true even if patients aged 56 to 65, 66 to 75, and older than 75 years were compared. It is conceivable that discrepant results among these studies, including ours, could be a reflection of differences in analyzed patient population. Through an entirely different approach, Juliusson et al evaluated the effect of age on outcomes for AML by using data for 2767 unselected patients with AML who were consecutively enrolled in the Swedish Acute Leukemia Registry.[11] They showed that intensive chemotherapy was associated with improved survival even for elderly patients, although it should be remembered that patients in that study were treated heterogeneously, and the choice of treatment must have been dependent on known and unknown confounding factors. Our study, in contrast, is advantageous in that the study population consisted of patients who were treated homogeneously regardless of age.

To summarize, we analyzed data for a large number of patients with AML aged 15 to 64 years who were treated uniformly in the context of clinical studies, and could not determine a specific age limit over which attenuation of treatment intensity is advisable. Our results justify the use of intensive chemotherapy without dose attenuation toward older but fit AML patients at least up to the age of 64.

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CONFLICT OF INTEREST DISCLOSURE

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

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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].

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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,

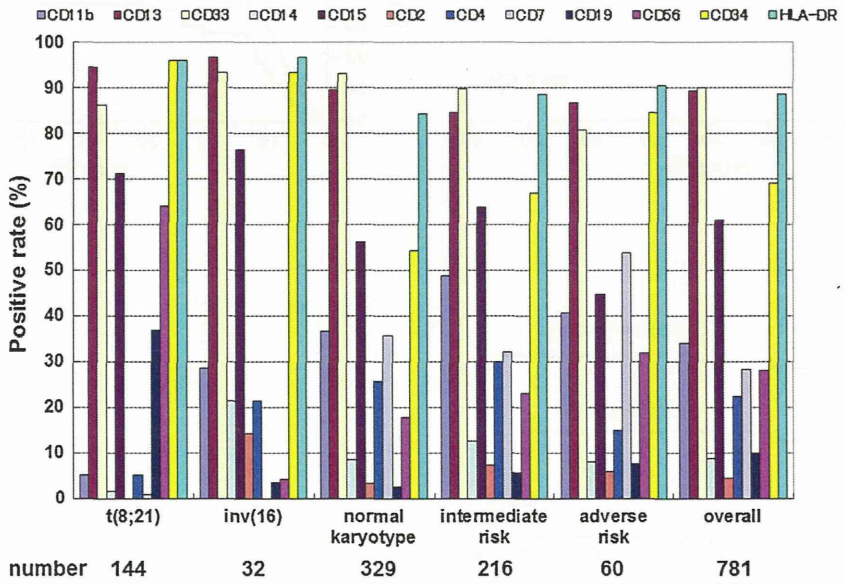

Fig. 1. Distribution of surface antigen expression. AML with t(8;21) is compared with other karyotypes of AML. Patients were classified into t(8;21), inv(16), normal karyotype, intermediate-risk (excluding normal karyotype), or adverse-risk group. Positive rates of the surface antigens CD11b, CD13, CD33, 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

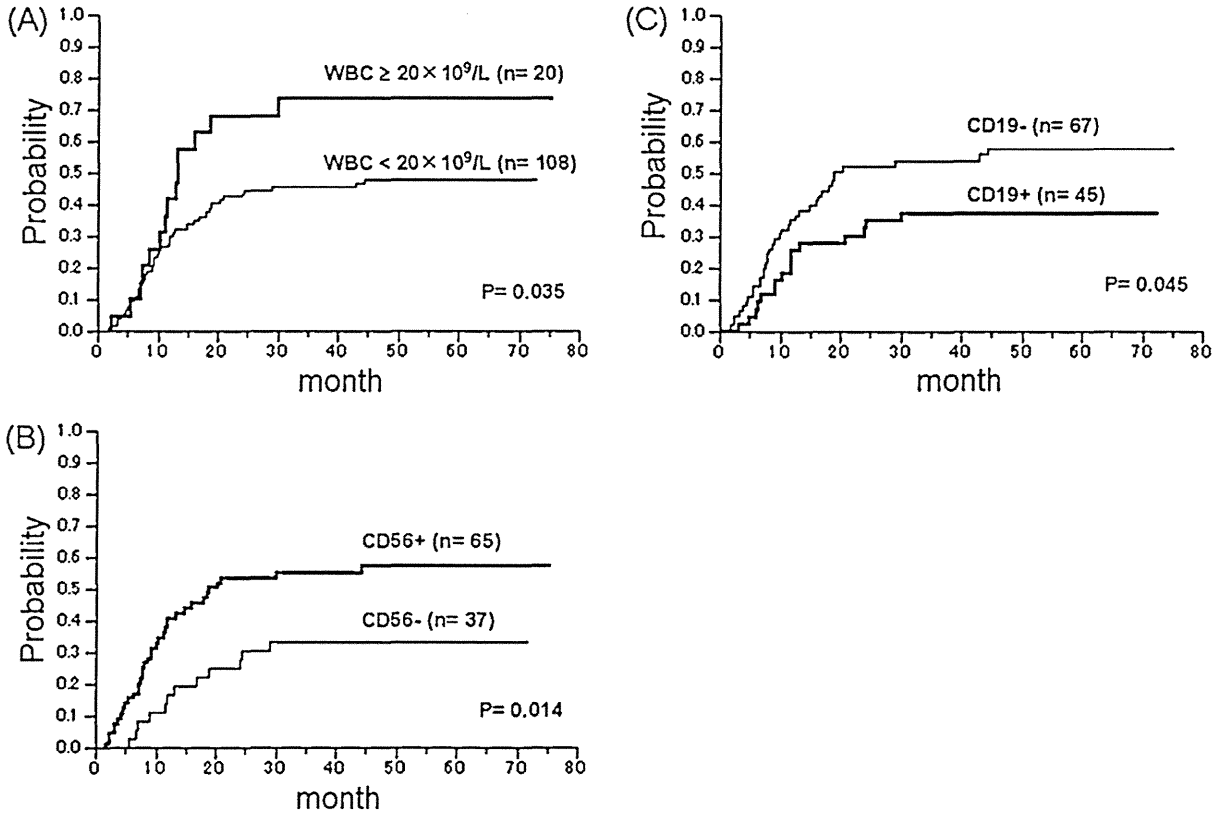

Fig. 2. Kaplan-Meier curves indicating cumulative incidence of relapse according to white blood cell count (WBC) (A), CD56 expression (B), and CD19 expression (C). Log-rank testing showed significant differences in the rate of relapse.

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 $\geq 20 \times 10^9$/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

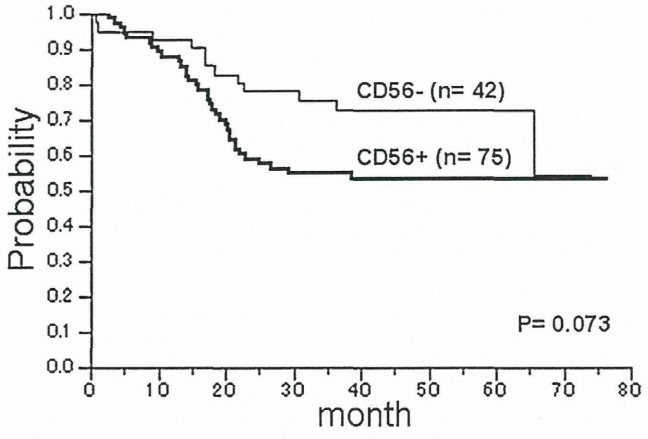

Fig. 3. Kaplan-Meier curves indicating overall survival (OS) according to CD56 expression. CD56 expression did not affect OS.

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 $\geq 20 \times 10^9$/L), CD56 positivity, and CD19 negativity were unfavorable factors for relapse. It has been previously shown that WBC $\geq 20 \times 10^9$/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 $\geq 20 \times 10^9$/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.

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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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CME/SAM

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

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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