PAS positivity of erythroid precursor cells is associated with a poor prognosis in newly diagnosed myelodysplastic syndrome patients

新たに診断された骨髄異形成症候群患者のPAS陽性赤芽球は不良な予後に関連する

増田　健太

Title

PAS positivity of erythroid precursor cells is associated with a poor prognosis in newly diagnosed myelodysplastic syndrome patients

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Running Head

PAS-positive erythroblasts in MDS patients

Type of Manuscript

Original article

Abstract

Myelodysplastic syndrome (MDS) is a group of clonal stem cell disorders characterized by hematopoietic insufficiency, and the accurate risk stratification of patients with MDS is essential for the selection of therapy. We herein conducted a retrospective cohort study to examine the prognostic value of periodic acid-Schiff (PAS) reaction-positive erythroblasts in MDS patients. We examined the PAS positivity of the bone marrow erythroblasts of 144 patients newly diagnosed with MDS; 26 (18.1%) of them had PAS-positive erythroblasts, whereas 118 (81.9%) did not. The PAS-positive group showed significantly poorer karyotypes in the revised International Prognostic Scoring System (IPSS-R), and higher scores in the age-adjusted IPSS-R (IPSS-RA) than the PAS-negative group. Furthermore, overall survival (OS) and leukemia-free survival (LFS) were significantly shorter in the PAS-positive group than in the PAS-negative group. Similar results were obtained when only the high and very-high risk groups were analyzed using IPSS-RA. Our retrospective study demonstrated that the PAS positivity of erythroblasts was an additional prognostic factor combined with other risk scores for OS and LFS in MDS, and the results obtained may contribute to proper clinical decision-making and rapid risk stratification.

Keywords

PAS-positive erythroblasts, Myelodysplastic syndrome, prognosis, International Prognostic Scoring System, revised International Prognostic Scoring System

Introduction

Myelodysplastic syndrome (MDS) is a heterogeneous group of clonal stem cell disorders characterized by peripheral cytopenia and dysplastic changes in bone marrow cells and is associated with a high risk of transformation to acute myeloid leukemia (AML)[1]. The annual incidence of MDS is 3-5/100,000, with age-specific rates increasing to >20/100,000 among individuals older than 70 years of age[2]. It is important to assess the disease risk at diagnosis using established prognostic scoring systems in order to estimate prognoses and make decisions including whether aggressive treatments, such as chemotherapy and potentially curative allogenic hematopoietic cell transplantation, are needed[3,4]. To date, various risk assessment systems have been proposed, among which the first widely adopted model was the International Prognostic Scoring System (IPSS). In IPSS, cytogenetic subgroups, marrow blast percentages, and the extent of cytopenia are incorporated into assessments of disease risk in primary untreated MDS patients[5]. The revised IPSS (IPSS-R) and age-adjusted IPSS-R (IPSS-RA) were subsequently proposed[6], and other prognostic systems have also been developed in order to precisely evaluate prognoses[7]. Since MDS is characterized by dysplastic changes in blood cells, morphological evidence of dysplasia upon a visual examination of bone marrow aspirates is essential for reaching a diagnosis[1,8]. Some cases of dysplasia are highly specific for the diagnosis of MDS: hypo-segmented mature neutrophils, the degranulation of neutrophils, micromegakaryocytes, and ringed sideroblasts[9–11].

These morphological features are also considered to be associated with prognosis, but are not included in IPSS, IPSS-R, or IPSS-RA[12,13]. Other features such as periodic acid-Schiff (PAS) reaction-positive erythroblasts are considered to be less specific dysplastic changes that may be observed in MDS[2,14]; however, the prognostic value of PAS-positive erythroblasts has not yet been evaluated.

Therefore, we herein conducted a retrospective cohort study and demonstrated the prognostic significance of PAS-positive erythroblasts in MDS patients.

Patients and Methods

Study Design

The purpose of the present study was to investigate whether the PAS positivity of erythroblasts in bone marrow aspirates is relevant to disease prognosis. The primary endpoint was the probability of overall survival (OS), which was defined as the time between diagnosis and death by any cause (for events) or the last follow-up (for censored patients), in MDS patients. The secondary endpoint was the probability of leukemia-free survival (LFS), which was defined as the time between diagnosis and documented leukemic transformation or death (for events), or the last follow-up (for censored patients). The date of leukemic transformation was defined as the time that blasts increased to 20% in either bone marrow or peripheral blood. In the definition of LFS, hematopoietic stem cell transplantation (HSCT) was considered to be a censored event. We reviewed electric medical records and collected laboratory data including absolute neutrophil counts, hemoglobin levels, platelet counts, myelograms, and cytogenetic data of bone marrow aspirates at diagnosis. We also recorded the exact date of the diagnosis of MDS with a bone marrow examination and the date of the last follow-up, death, or leukemic transformation for each case. This retrospective cohort study protocol was approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine, and was performed in accordance with the Declaration of Helsinki.

Patients and Risk Assessment

Patients whose bone marrow samples were submitted to the Department of Clinical Laboratory of Kyoto University Hospital for the diagnosis of MDS between 2007 and 2016 were enrolled. Patients who had not received disease-altering treatments for MDS were eligible for inclusion in the present study. We excluded patients with the criteria outlined in the patient flow diagram (Fig. 1). Patients with 20-29% blasts in bone marrow smears (RAEB-t) and with chromosome inv(16) were excluded from the analysis because these entities were classified as AML by the WHO 2008 classification[15]. We also excluded patients with chronic myelomonocytic leukemia (CMMoL), which was categorized into myelodysplastic/myeloproliferative neoplasms (MDS/MPN) by the WHO 2008 classification[15]. In the present study, based on the WHO 2008 classification, we carefully excluded cases that matched the criterion AML-M6: 50% or more of all nucleated cells (ANC) were erythroblasts and 20% or more of the remaining cells (non-erythroid cells: NEC) were myeloblasts. We reconfirmed diagnoses according to the WHO 2008 classification and calculated prognostic scores using IPSS-RA[16]. The percentage of blasts in the bone marrow was calculated using ANC or NEC as the denominator[2]. Cytogenetic aberrations were evaluated following the International Working Group on the MDS Cytogenetics (IWGMC) consensus guidelines in order to calculate accurate IPSS-RA scores[17].

Morphological Evaluation

We reviewed bone marrow aspirate smears that had been stained using the May-Grünwald-Giemsa stain and PAS reaction, and two independent specialists assessed the PAS positivity of erythroblasts using a visual inspection. Cases that varied in evaluations of PAS positivity between two experts were carefully assessed under a multi-observer microscope.

We performed the PAS reaction using a method modified from that of McManus[18] and Oguro[19], the details of which are as follows:

(1) Formalin-fixed preparations of bone marrow aspirates are oxidized in 1% periodic acid solution for 10 minutes. (2) Rinse in water for 10 minutes. (3) Place in Schiff reagent for 40 minutes. (4) Rinse in water for 10 minutes. (5) Counterstain in Mayer's hematoxylin for 25 minutes. (6) Wash in lukewarm water for 10 minutes.

We inspected at least 500 erythroblasts of bone marrow aspirate slides and categorized the slides with no PAS-positive erythroblasts as negative samples. The PAS reaction displayed a coarse positive pattern in immature erythroblasts (Fig. 2a) and a diffuse positive pattern in intermediate and mature erythroblasts (Fig. 2b), which were both categorized as positive samples. We considered erythroblasts, the color of which was a brighter red than the cytoplasm of neutrophils, as positive cells. Samples with one positive cell among 500 erythroblasts (cut-off = 0.2%) belonged to the PAS-positive group. If only one positive cell was found among 500 erythroblasts, we examined all other erythroblasts and searched for at least three positive cells in order to avoid false-positive cases.

Statistical Analysis

We performed comparisons of characteristics between PAS-positive and PAS-negative patients enrolled between 2007 and 2016 using the Mann-Whitney *U* test for continuous variables and the chi-squared test for categorical variables.

Kaplan-Meier analyses were employed to assess the probability of survival for those enrolled between 2007 and 2012, and survival curves were compared by Log-rank tests (univariate analysis). Cox's regression proportional hazards model was employed in order to identify independent factors associated with OS and LFS (multivariate analysis). We selected 8 factors considered to influence the prognosis of MDS, i.e. the PAS positivity of erythroblasts, gender, age, peripheral blood cytopenia, bone marrow blast percentage, bone marrow erythroblast percentage, IPSS-R karyotype, and IPSS-RA risk groups.

In the analysis of OS and LFS, patients who were still alive were censored on 31st December 2014. Data were analyzed using EZR (version 1.36)[20] and StatMate (ATMS Co., Ltd., Tokyo, Japan). *P* values were two-sided, and $P < 0.05$ was considered to be significant.

Results

Patient Characteristics

We examined the PAS positivity of the bone marrow erythroblasts of 144 patients newly diagnosed with MDS in our institution between 2007 and 2016. The median age of our cohort at diagnosis was 66.8 years (range: 2.6 - 90.7), and 90 patients (62.5%) were male. Among 26 PAS-positive cases, the median percentage of the PAS positive erythroblasts was 2.6% (range, 0.4 to 18.0%; the distribution is shown in Supplementary Fig. S1).

Detailed patient characteristics are shown in Table 1. We divided patients into two groups according to the PAS positivity of bone marrow aspirate samples. Twenty-six (18.1%) patients had PAS-positive erythroblasts (PAS-positive group), whereas 118 (81.9%) did not (PAS-negative group). No significant differences were observed in gender, age, the absolute neutrophil count, or platelet count between these groups.

In contrast, the median values of hemoglobin (77 g/L vs 93 g/L), bone marrow blast percentage (7.0% vs 2.8%), and bone marrow erythroblast percentage (42.6% vs 31.6%) between PAS-positive and PAS-negative patients were significantly different. The PAS-positive group showed significantly higher karyotype scores in IPSS-R than the PAS-negative group. Eighteen out of 26 PAS-positive patients (69.2%), in contrast to only 7 out of 118 (5.9%) in the PAS-negative group, had a very poor karyotype (complex karyotype with 4 or more abnormalities) according to the IPSS-R scoring system (Table 2)[16]. Furthermore, the PAS-positive group showed significantly higher risk scores in IPSS-RA than the PAS-negative group (P value < 0.0001) (Table 1). The PAS-positive group showed significantly higher risk MDS subtypes (RAEB-1 or RAEB-2) in the WHO 2008 classification (16 out of 26 cases). These results were in agreement with the bone marrow blast percentage of the PAS-positive group being significantly higher than that of the PAS-negative group.

Based on the WHO 2008 classification, the denominator used to calculate the blast% of samples with 50% or more erythroblasts was NEC[2]; therefore, 6 patients were excluded as AML-M6 (Fig. 1). The PAS-positive ratio of the cases categorized as AML-M6 in the present study was 83.3% (5 out of 6 cases). However, based on the definition of the WHO 2016 classification, these cases were included in MDS[21]. We analyzed the characteristics of 150 patients based on the WHO 2016 classification (Table S1), which were similar to those shown in Table 1. We also stratified IPSS and IPSS-R risk scores based on the WHO 2008 classification and showed the results obtained (Table S2) and those based on the WHO 2016 classification (Table S3); the PAS-positive groups showed poorer karyotypes and higher risks than the PAS-negative groups.

Evaluation of Prognostic Factors by Univariate Analyses

Eighty-three MDS patients diagnosed between 2007 and 2012 were enrolled for prognostic

analyses (Fig. 1), and the impacts of various clinical factors on survival were analyzed (Table 3). Based on the electric medical records available at the time of evaluation, the numbers of patients who received hematopoietic growth factors, immunosuppressive drugs, hypomethylating agents, lenalidomide, intensive chemotherapies, stem cell transplantation, and supportive care only were 6, 5, 6, 1, 6, 21, and 38, respectively (some patients received multiple therapies).

Patients with PAS-positive erythroblasts in bone marrow smears had a median OS of 456 days, while that of patients without PAS-positive erythroblasts was 1721 days (*P* value = 0.0047), with a hazard ratio of 2.804. A significant effect was also observed in LFS with a hazard ratio of 3.531 (*P* value = 0.0008). Kaplan-Meier analyses showed that PAS positivity was associated with significantly poor prognoses for OS and LFS (Fig. 3). When we analyzed only those in the high IPSS-RA risk groups ("Very high" or "High"), PAS positivity still correlated with shorter OS and LFS (*P* values of 0.0155 and 0.0429, respectively; Fig. 4). Univariate analyses revealed that other variables also had significant impacts on OS and LFS by the Log-rank test (Table 3): bone marrow blast percentage (*P* values = 0.0150 and 0.0020, respectively), IPSS-R karyotypes (*P* values = 0.0069 and 0.0031, respectively), and IPSS-RA risk groups (*P* value = 0.0004 and *P* value < 0.0001, respectively). IPSS and IPSS-R also had significant impacts on OS and LFS in the univariate analysis (Table S4). However, the bone marrow erythroblast percentage did not have a significant impact on OS or LFS.

Assessment of Prognostic Factors by Multivariate Analyses

We applied Cox's multivariate regression analysis to clarify whether the inclusion of PAS positivity increases the predictive value of various prognostic factors. We selected three variables – PAS positivity, bone marrow blast percentage, and the IPSS-R karyotype, which had significant impacts on OS and LFS in the univariate analyses (Table 3). In this model, none of these variables had a significant impact on OS or LFS (Table 4).

Discussion

In the present study, we showed that most PAS-positive patients had poorer karyotypes in IPSS-R; therefore, the calculated IPSS-RA score was high in these scoring systems (Table 1). We also demonstrated that MDS patients with PAS-positive erythroblasts had shorter median OS and LFS than those without PAS-positive erythroblasts in a univariate analysis (Table 3 and Fig. 3). When patients with high IPSS-RA scores, namely, "Very high" or "High", were divided by PAS positivity and OS and LFS were analyzed using the Kaplan-Meier method, the two curves significantly differed (Fig. 4), suggesting that IPSS-RA combined with PAS positivity discriminates patients with a poorer prognosis than that predicted by IPSS-RA only.

In the WHO 2008 classification[15] and its revised version[21], AML with myelodysplasia-related changes (AML-MRC) was recognized as a specific entity separate from "AML not otherwise specified" (AML-NOS) based on the presence of multilineage dysplasia (MLD), MDS-related cytogenetics, or a history of MDS. Previous studies demonstrated that AML patients with MLD had a poorer prognosis than those without MLD[22,23]. Furthermore, MDS patients with trilineage dysplasia were reported to more often have unfavorable cytogenetic profiles, and a correlation was found between the presence or absence of dysplastic features and cytogenetic subgroups[24]. Although dysgranulopoiesis has been suggested to be associated with lower complete remission rates in *de novo* AML[25], limited information is currently available on the relationship between the type of dysplastic change and the prognosis of MDS.

The PAS reaction detects intracellular polysaccharides such as glycogen, glycoproteins, and glycolipids. Neutrophils, megakaryocytes, and platelets are positive for PAS due to glycogen, and eosinophils and basophils presumably due to other carbohydrates[26]. Normal human erythroblasts are expected to be negative, and PAS positivity in dysplastic erythroblasts indicates abnormal carbohydrate metabolism in these cells. Erythroblasts not only from patients with thalassemia[27], megaloblastic anemia[28], MDS, and erythroleukemia[29], but also from normal fetal blood showed PAS positivity[30]. However, the pathophysiological significance of PAS-positive erythroblasts has not yet been clarified. The present results suggest that although the PAS positivity of erythroblasts is not necessarily disease-specific dysplasia, once the diagnosis of MDS has been established by other means, the presence of PAS-positive erythroblasts indicates a poor prognosis. We speculate that the reason for the worse prognosis of the PAS-positive group in our cohort was the significantly poorer karyotype (particularly complex karyotypes with 4 or more abnormalities) than the PAS-negative group (Table 2).

The PAS-positive group showed a significantly higher bone marrow erythroblast percentage than the PAS-negative group (42.6% vs 31.6%, *P* value = 0.0252, Table 1); however, univariate analyses revealed that the median OS and LFS of those with bone marrow erythroblasts ≥50% did not significantly differ from those with erythroblasts <50% (Table 3). We speculate that not

the high percentage of erythroblasts but the PAS positivity of erythroblasts contributed to the poor prognosis of MDS patients.

In the multivariate analysis, we selected three variables that had significant impacts on OS and LFS in univariate analyses. In this model, none of these variables had a significant impact on OS, whereas PAS positivity and bone marrow blast percentage had a slight impact on LFS. Since the PAS positivity of erythroblasts strongly correlated with the karyotype score using the chi-squared test (Table 1), the simultaneous inclusion of PAS positivity and karyotype scores in the multivariate model may have resulted in multicollinearity and weakened their impact on survival.

Some limitations need to be addressed. The present study was a retrospective analysis of MDS patients from one hospital, and, thus, our results need to be verified in a prospective study. Furthermore, our cohort only included 83 patients applicable to the multivariate analysis, and only three variables were selected for Cox's regression proportional hazard analysis. We need to recruit more MDS patients and perform the same examination in order to confirm the results of the present study.

In conclusion, our retrospective study demonstrated that the PAS positivity of erythroblasts is an additional prognostic variable combined with other risk scores for OS and LFS in MDS. To the best of our knowledge, this is the first study to examine the prognostic impact of PAS-positive erythroblasts, and the results obtained may contribute to proper clinical decision-making and rapid risk stratification.

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Conflict of interest

No conflicts of interest to disclose.

Figures

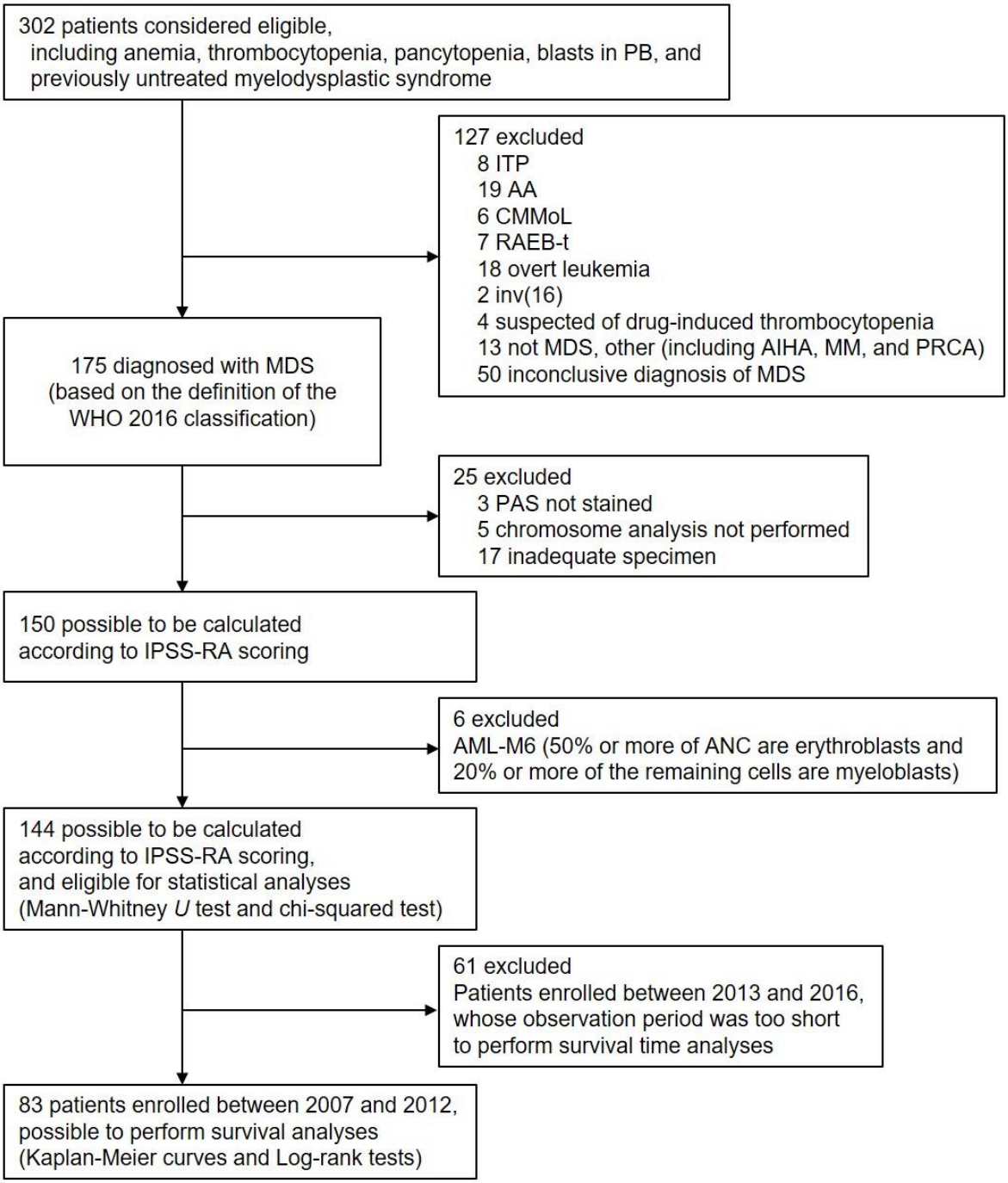

Fig.1 Exclusion and inclusion criteria

We performed statistical analyses (the Mann-Whitney *U* test and chi-squared test) using 144 patients enrolled between 2007 and 2016. We then excluded patients enrolled between 2013 and 2016 and performed survival analyses.

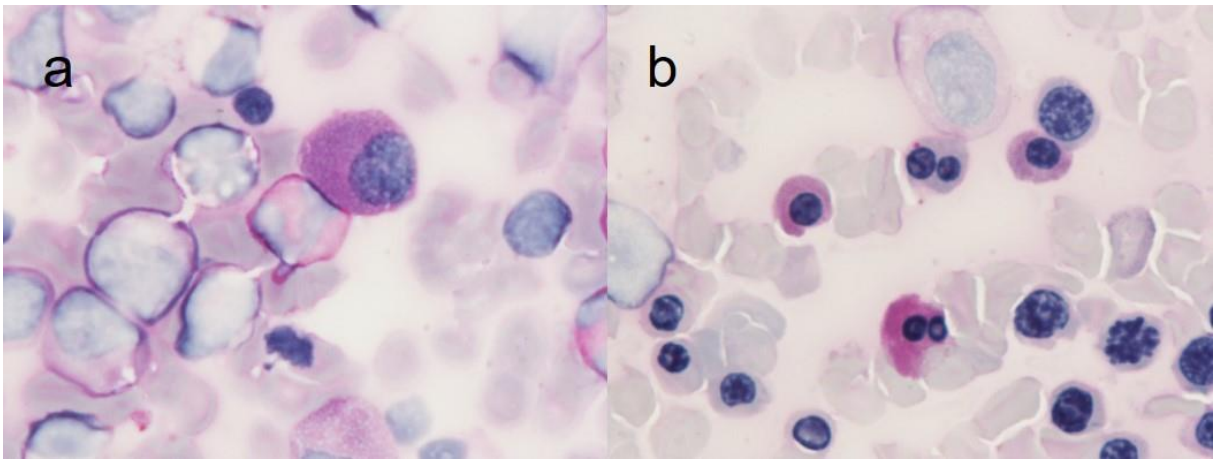

Fig. 2 PAS positivity of erythroid precursor cells
The PAS reaction displays a coarse positive pattern in immature erythroblasts (a) and a diffuse pattern in intermediate and mature erythroblasts (b).

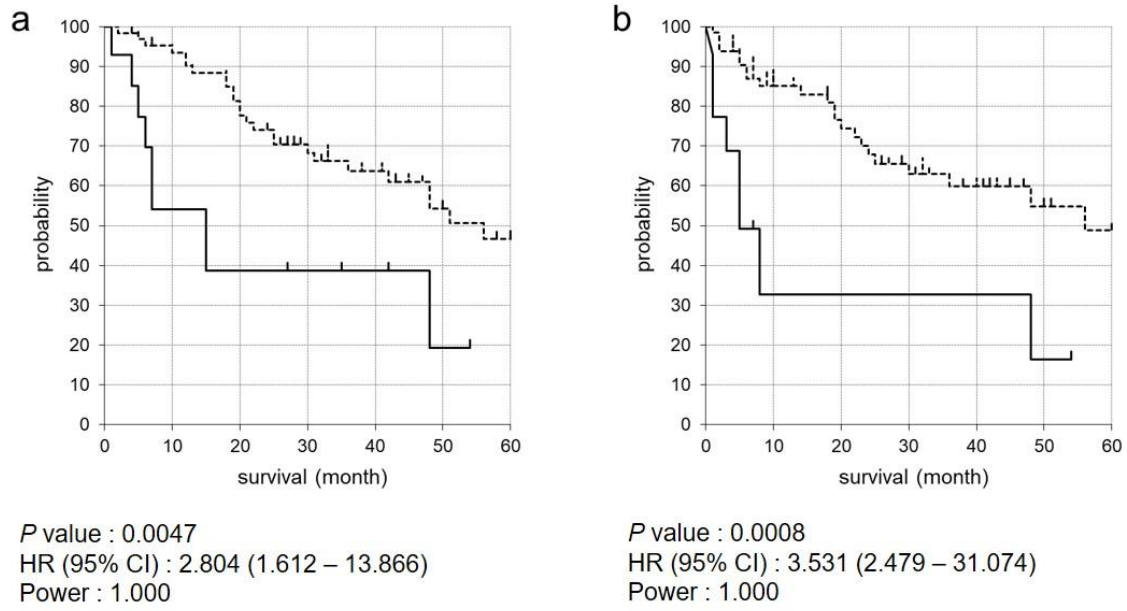

Fig. 3 Kaplan-Meier survival curves of MDS patients with the PAS positivity of erythroblasts
Overall survival (Fig. 3a) and leukemia-free survival (Fig. 3b) of 83 MDS patients stratified by PAS positivity. PAS-positive (solid line, n = 14) and PAS-negative (dotted line, n = 69).

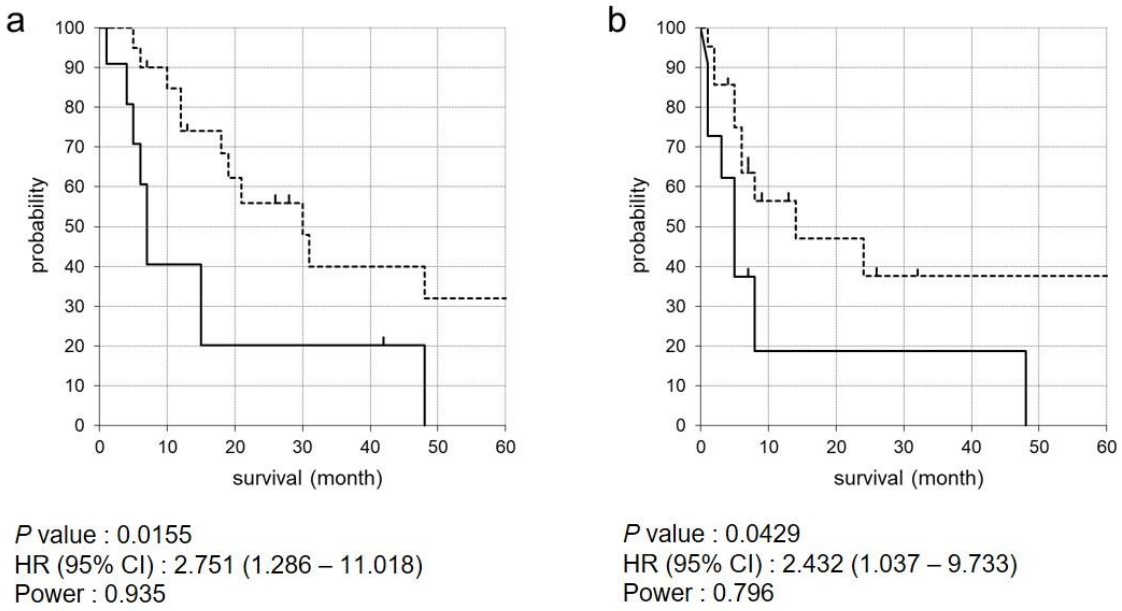

Fig. 4 Kaplan-Meier survival curves of MDS patients with the PAS positivity of erythroblasts among high IPSS-RA groups ("Very high" or "High")

Overall survival (Fig. 4a) and leukemia-free survival (Fig. 4b) of 32 MDS patients with high IPSS-RA scores stratified by PAS positivity. PAS-positive (solid line, n = 11) and PAS-negative (dotted line, n = 21).

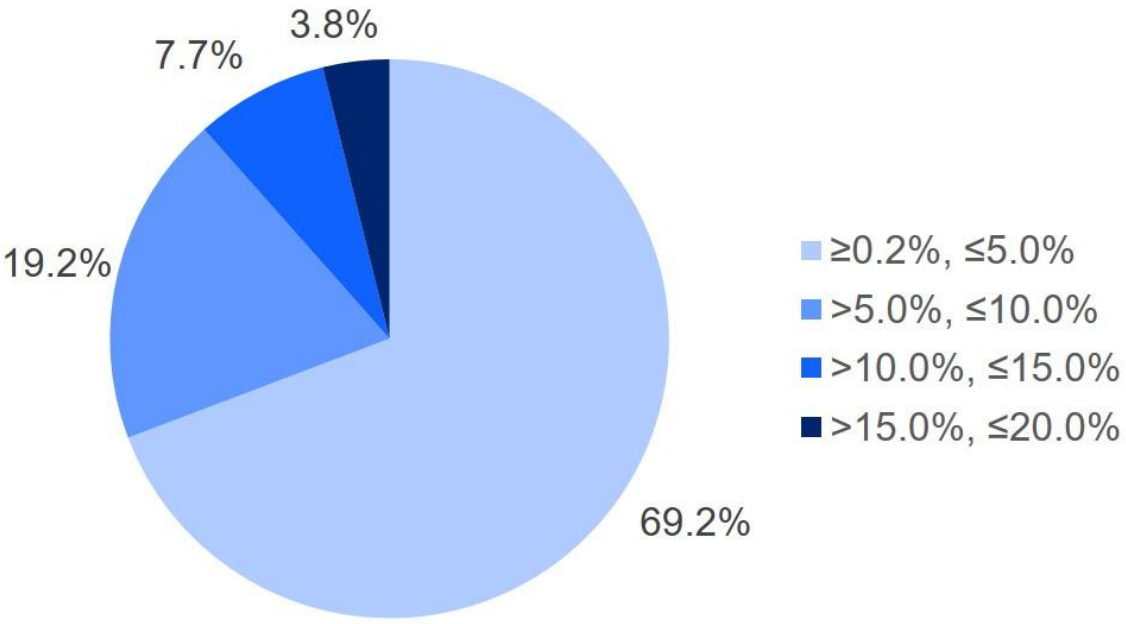

Fig.S1 Distribution of percentage of the PAS-positive erythroblasts among 26 PAS-positive MDS cases.

Table 1 Laboratory features at the time of diagnosis (based on the WHO 2008 classification)

	Total	PAS-positive	PAS-negative	*P* value
Patients, no.	144	26	118	
Gender, no. (%)				
Male	90 (62.5%)	13 (50.0%)	77 (65.3%)	0.15 †
Female	54 (37.5%)	13 (50.0%)	41 (34.7%)	
Age, y	66.8 (2.6 - 90.7)	64.6 (27.2 - 85.4)	67.1 (2.6 - 90.7)	0.66 *
Absolute neutrophil count ($\times 10^9$/L)	1.4 (0.2 - 22.6)	1.4 (0.3 - 6.1)	1.3 (0.2 - 22.6)	0.75 *
Hemoglobin (g/L)	89 (48 - 153)	77 (48 - 151)	93 (48 - 153)	0.0238 *
Platelet count ($\times 10^9$/L)	91 (8 - 745)	67 (9 - 552)	94 (8 - 745)	0.17 *
Bone marrow blast percentage (%)	2.9 (0.0 - 19.6)	7.0 (0.4 - 19.6)	2.8 (0.0 - 17.6)	0.0032 *
Bone marrow erythroblast percentage (%)	33.2 (0.8 – 85.6)	42.6 (15.2 – 85.6)	31.6 (0.8 – 71.2)	0.0252 *
IPSS-R karyotype, no. (%)				
Very good	10 (6.9%)	1 (3.8%)	9 (7.6%)	< 0.0001 †
Good	59 (41.0%)	4 (15.4%)	55 (46.6%)	
Intermediate	30 (20.8%)	2 (7.7%)	28 (23.7%)	
Poor	20 (13.9%)	1 (3.8%)	19 (16.1%)	
Very poor	25 (17.4%)	18 (69.2%)	7 (5.9%)	
IPSS-RA risk groups, no. (%)				
Very low	11 (7.6%)	1 (3.8%)	10 (8.5%)	< 0.0001 †
Low	42 (29.2%)	2 (7.7%)	40 (33.9%)	
Intermediate	33 (22.9%)	2 (7.7%)	31 (26.3%)	
High	23 (16.0%)	4 (15.4%)	19 (16.1%)	
Very high	35 (24.3%)	17 (65.4%)	18 (15.3%)	
WHO 2008 classification, no (%)				0.0346 †
RCUD or RARS	27 (18.8%)	1 (3.8%)	26 (22.0%)	
RCMD	62 (43.1%)	9 (34.6%)	53 (44.9%)	
MDS-U	2 (1.4%)	0 (0.0%)	2 (1.7%)	
RAEB-1	28 (19.4%)	7 (26.9%)	21 (17.8%)	
RAEB-2	25 (17.4%)	9 (34.6%)	16 (13.6%)	

'*: Data are presented as a median (range) and analyzed using the Mann-Whitney *U* test for continuous variables.

'†: Data are presented as n (percentage) and analyzed using the chi-squared test for categorical variables

Table 2 IPSS-R karyotype at the time of diagnosis

	Total	PAS-positive	PAS-negative
Total	144	26	118
Very good, no. (%)	10 (100 %)	1 (10.0%)	9 (90.0%)
-Y	7	1	6
del(11q)	3	0	3
Good, no. (%)	59 (100 %)	4 (6.8%)	55 (93.2%)
Normal	56	4	52
del(5q)	0	0	0
del(12p)	0	0	0
del(20q)	3	0	3
double including del(5q)	0	0	0
Intermediate, no. (%)	30 (100%)	2 (6.7%)	28 (93.3%)
del(7q)	2	0	2
+8	4	0	4
+19	0	0	0
i(17q)	0	0	0
any other single or double independent clones	24	2	22
Poor, no. (%)	20 (100 %)	1 (5.0%)	19 (95.0%)
-7	2	0	2
inv(3)/t(3q)/del(3q)	0	0	0
double including -7/del(7q)	3	0	3
complex karyotype (3 abnormalities)	15	1	14
Very poor, no. (%)	25 (100 %)	18 (72.0%)	7 (28.0%)
complex karyotype (4 or more abnormalities)	25	18	7

Table 3 Survival of MDS patients according to relevant clinical factors

		Overall survival			Leukemia-free survival		
	N	MST (days)	*P* value	HR (95% CI)	MST (days)	*P* value	HR (95% CI)
PAS positivity							
Positive	14	456	0.0047	2.804 (1.612 - 13.866)	170	0.0008	3.531 (2.479 - 31.074)
Negative	69	1721			1721		
Gender							
Male	54	1569	0.90	1.043 (0.524 - 2.080)	2385	0.96	1.021 (0.486 - 2.145)
Female	29	1489			1476		
Age							
≥ 70 y	35	1489	0.89	1.048 (0.515 - 2.142)	1489	0.70	1.145 (0.559 - 2.367)
< 70 y	48	1478			1476		
Peripheral blood cytopenia							
2 or 3 lineages	57	1489	0.60	1.214 (0.592 - 2.480)	1489	0.52	1.286 (0.604 - 2.727)
0 or 1 lineage	26	NR			NR		
Bone marrow blast percentage							
≥ 5.0%	34	790	0.0150	2.303 (1.189 - 5.005)	756	0.0020	2.799 (1.602 - 8.162)
< 5.0%	49	1721			2385		
Bone marrow erythroblast percentage							
≥ 50.0%	21	1476	0.87	1.064 (0.489 – 2.325)	1479	0.73	0.871 (0.396 – 1.920)
< 50.0%	62	1489			1489		
IPSS-R karyotype							
Very poor or Poor	24	577	0.0069	2.415 (1.348 - 6.539)	267	0.0031	2.736 (1.574 - 9.321)
Intermediate, Good, or Very good	59	1721			1721		
IPSS-RA risk groups							
Very high or High	32	577	0.0004	3.061 (1.786 - 7.741)	252	< 0.0001	3.861 (2.520 - 13.202)
Intermediate, Low, or Very low	51	2385			2385		

MST: median survival time

NR: not reached

Table 4 Multivariate analysis of OS and LFS in MDS patients

	Overall survival			Leukemia-free survival		
	HR	95% CI	*P* value	HR	95% CI	*P* value
PAS positivity	2.009	0.854 – 4.725	0.11	2.465	0.925 – 6.566	0.07
Bone marrow blast percentage (5.0% or more)	1.776	0873 – 3.612	0.11	2.212	0.978 – 5.002	0.06
IPSS-R karyotype (Very poor or Poor)	1.656	0.761 – 3.604	0.20	1.460	0.572 – 3.726	0.43

HR: hazard ratio

95% CI: 95% confidence interval

Table S1 Laboratory features at the time of diagnosis (based on the WHO 2016 classification)

	Total	PAS-positive	PAS-negative	*P* value
Patients, no.	150	31	119	
Gender, no. (%)				
Male	94 (62.7%)	16 (51.6%)	78 (65.5%)	0.15 †
Female	56 (37.3%)	15 (48.4%)	41 (34.5%)	
Age, y	66.8 (2.6 – 90.7)	65.8 (27.2 – 88.2)	67.2 (2.6 – 90.7)	0.80 *
Absolute neutrophil count ($\times 10^9$/L)	1.3 (0.2 – 22.6)	1.3 (0.2 – 6.1)	1.3 (0.2 – 22.6)	0.82 *
Hemoglobin (g/L)	88 (48 – 153)	75 (48 - 151)	93 (48 – 153)	0.0084 *
Platelet count ($\times 10^9$/L)	91 (8 - 745)	88 (9 - 552)	93 (8 - 745)	0.31 *
Bone marrow blast percentage (%)	2.3 (0.0 – 18.0)	3.6 (0.2 – 18.0)	2.0 (0.0 – 17.6)	0.0172 *
Bone marrow erythroblast percentage (%)	34.2 (0.8 – 92.4)	48.0 (15.2 – 92.4)	32.0 (0.8 – 71.2)	0.0019 *
IPSS-R karyotype, no. (%)				
Very good	10 (6.7%)	1 (3.2%)	9 (7.6%)	< 0.0001 †
Good	60 (40.0%)	5 (16.1%)	55 (46.2%)	
Intermediate	30 (20.0%)	2 (6.5%)	28 (23.5%)	
Poor	20 (13.3%)	1 (3.2%)	19 (16.0%)	
Very poor	30 (20.0%)	22 (71.0%)	8 (6.7%)	
IPSS-RA risk groups, no. (%)				
Very low	15 (10.0%)	1 (3.2%)	14 (11.8%)	< 0.0001 †
Low	44 (29.3%)	2 (6.5%)	42 (35.3%)	
Intermediate	32 (21.3%)	3 (9.7%)	29 (24.4%)	
High	24 (16.0%)	6 (19.4%)	18 (15.1%)	
Very high	35 (23.3%)	19 (61.3%)	16 (13.4%)	

Table S2 Risk stratification of IPSS and IPSS-R (based on the WHO 2008 classification)

	Total	PAS-positive	PAS-negative	*P* value
Patients, no.	144	26	118	
IPSS karyotype, no. (%)				
Good	66 (45.8%)	5 (19.2%)	61 (51.7%)	< 0.0001 †
Intermediate	30 (20.8%)	2 (7.7%)	28 (23.7%)	
Poor	48 (33.3%)	19 (73.1%)	29 (24.6%)	
IPSS risk groups, no. (%)				
Low	20 (13.9%)	1 (3.8%)	19 (16.1%)	< 0.0001 †
Int-1	69 (47.9%)	4 (15.4%)	65 (55.1%)	
Int-2	38 (26.4%)	14 (53.8%)	24 (20.3%)	
High	17 (11.8%)	7 (26.9%)	10 (8.5%)	
IPSS-R risk groups, no. (%)				
Very low	7 (4.9%)	1 (3.8%)	6 (5.1%)	< 0.0001 †
Low	50 (34.7%)	2 (7.7%)	48 (40.7%)	
Intermediate	31 (21.5%)	1 (3.8%)	30 (25.4%)	
High	22 (15.3%)	5 (19.2%)	17 (14.4%)	
Very high	34 (23.6%)	17 (65.4%)	17 (14.4%)	

Table S3 Risk stratification of IPSS and IPSS-R (based on the WHO 2016 classification)

	Total	PAS-positive	PAS-negative	*P* value
Patients, no.	150	31	119	
IPSS karyotype, no. (%)				
Good	67 (44.7%)	6 (19.4%)	61 (51.3%)	< 0.0001 †
Intermediate	30 (20.0%)	2 (6.5%)	28 (23.5%)	
Poor	53 (35.3%)	23 (74.2%)	30 (25.2%)	
IPSS risk groups, no. (%)				
Low	23 (15.3%)	1 (3.2%)	22 (18.5%)	< 0.0001 †
Int-1	71 (47.3%)	6 (19.4%)	65 (54.6%)	
Int-2	46 (30.7%)	21 (67.7%)	25 (21.0%)	
High	10 (6.7%)	3 (9.7%)	7 (5.9%)	
IPSS-R risk groups, no. (%)				
Very low	10 (6.7%)	1 (3.2%)	9 (7.6%)	< 0.0001 †
Low	54 (36.0%)	3 (9.7%)	51 (42.9%)	
Intermediate	28 (18.7%)	1 (3.2%)	27 (22.7%)	
High	24 (16.0%)	7 (22.6%)	17 (14.3%)	
Very high	34 (22.7%)	19 (61.3%)	15 (12.6%)	

Table S4 Survival of MDS patients according to IPSS and IPSS-R risk groups

		Overall survival			Leukemia-free survival		
	N	MST (days)	*P* value	HR (95% CI)	MST (days)	*P* value	HR (95% CI)
IPSS karyotype							
Poor	25	577	0.0041	2.528 (1.433 - 6.751)	267	0.0007	3.089 (1.906 - 11.241)
Intermediate or Good	58	2385			2385		
IPSS risk groups							
High or Int-2	31	652	0.0027	2.627 (1.470 - 6.278)	252	0.0004	3.205 (1.994 - 10.803)
Int-1 or Low	52	2385			2385		
IPSS-R risk groups							
Very high or High	31	548	< 0.0001	3.482 (2.163 - 9.768)	252	< 0.0001	4.890 (4.144 - 24.699)
Intermediate, Low, or Very low	52	2385			2385		