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# A systematic classification of megakaryocytic dysplasia and its impact on prognosis for patients with myelodysplastic syndromes

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## Abstract

**Background:** Dys-megakaryopoiesis is defined as  $\geq 10\%$  of dysplastic megakaryocytes in bone marrow smears by the World Health Organization. However, concordance rates for dysplastic megakaryocytes between different observers is low and, consequently, evaluation of dysmegakaryopoiesis is also often discordant.

**Results:** We performed CD41 immune staining and proposed a systematic classification of dys-megakaryopoiesis on bone marrow films: (1) micro-megakaryocytes ( $< 12\ \mu\text{m}$ ); (2) micro-megakaryocytes ( $12\text{--}40\ \mu\text{m}$ ) with 1 nucleus; (3) micro-megakaryocytes ( $12\text{--}40\ \mu\text{m}$ ) with 2 nuclei; (4) micro-megakaryocytes ( $12\text{--}40\ \mu\text{m}$ ) with multiple (more than 2) nuclei; (5) dysplastic megakaryocytes ( $\geq 40\ \mu\text{m}$ ) with 1 nucleus; (6) dysplastic megakaryocytes ( $\geq 40\ \mu\text{m}$ ) with 2 nuclei; and (7) dysplastic megakaryocytes ( $\geq 40\ \mu\text{m}$ ) with multiple (more than 2) nuclei. Further, we evaluated the prognostic impact of micro-megakaryocytes and dysplastic mono-nucleated megakaryocytes on MDS patients. The best discriminator cut-off point for each group was determined by the minimal *P* value approach. In multivariate analyses micro-megakaryocytes  $\geq 25\%$  and dysplastic mono-nucleated megakaryocytes  $\geq 30\%$  were independent adverse prognostic factors (hazard ratio [HR] = 1.58 [95 % confidence interval [CI], 1.11, 2.23]; *P* = 0.010 and 1.53 [1.09, 2.16]; *P* = 0.014).

**Conclusions:** Our data suggest integration of micro-megakaryocytes and dysplastic mono-nucleated megakaryocytes improve predictive accuracy of the international prognostic scoring system-revised (IPSS-R) scoring system.

**Keywords:** Dysplastic megakaryocytes, MDS, Immunocytochemistry, Prognosis

## Background

Myelodysplastic syndromes (MDS) are a heterogeneous group of bone marrow neoplasms with variable clinical courses and prognoses [1]. Distinguishing the different form of MDS is important for accurate diagnosis, predicting outcomes and directing therapy. Several variables are used to distinguish different forms of MDS including

morphology, histology, blood and bone marrow cell counts, cytogenetics and molecular genetics. Despite recent advances, cytological features in blood films and bone marrow aspirates and histological findings in trephine biopsies remain key elements for diagnosing MDS [2, 3]. Among the histological parameters of MDS, multilineage dysplasia and percent bone marrow blasts are associated with unfavorable outcomes [4–7].

Megakaryocyte morphology is another important component in classifying MDS. The World Health Organization (WHO) 2008 classification defines dysmegakaryopoiesis as micro-megakaryocytes, hypo-lobed,

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or non-lobed nuclei in megakaryocytes of all sizes and multiple, widely-separated nuclei [8]. Although this definition of dys-megakaryopoiesis is potentially useful, there is no precise definition of micro-megakaryocytes in the WHO classification. Consequently it is not surprising that there is low concordance amongst observers for micro-megakaryocytes in bone marrow samples from persons with MDS [4, 9–12].

Megakaryocytes express surface CD41/CD61 and/or CD42b and CD42a [13, 14]. The glycoprotein (Gp) IIb (CD41), which has been considered a specific marker for the megakaryocyte lineage [15], can be detected during megakaryocytic differentiation at a stage of a late megakaryocytic progenitor [16–18]. Consequently, using CD41 to identify megakaryocytes may be a better way to define dysplastic megakaryocytes than Wright-Giemsa or May-Grünwald-Giemsa staining. We used CD41 immune staining to identify megakaryocytes and assess if they were dysplastic in bone marrow smears from persons with MDS. Further, we tried to describe the morphological features of megakaryocytic dysplasia by developing a systematic classification of megakaryocytic dysplasia and analyze the impact of our classification of dys-megakaryopoiesis on determining the prognosis of persons with MDS.

## Methods

### Study cohort

The study was approved by the ethics committees of the institute of hematology, Chinese Academy Of Medical Sciences (CAMS) and Peking Union Medical College (PUMC) according to guidelines of the declaration of Helsinki. In this retrospective analysis, the study cohort included 422 consecutive new-diagnosed subjects that were seen at the Institute of Hematology and Blood Disease Hospital, Chinese Academy of Medical Sciences from January, 2000 to April, 2014. 8 subsequently received a haematopoietic cell transplant, 14, decitabine, 45, other chemotherapy and the remainder cyclosporine or thalidomide and best supportive care. Cases were re-reviewed by two blinded pathologists (W Cui and W Cai) and classified using the 2008 WHO criteria [2]. Subjects with suspected therapy-related MDS were excluded as the clinical course was typically progressive and treatment with conventional therapy was usually associated with a poor prognosis [19]. Furthermore, there was no Down Syndrome patient in the cohort. Follow-up data were available for 370 subjects (88 %). Date of last follow-up was December 15, 2014 or date of last contact. Median follow-up was 22 months (range 1–180 months). Subjects with lower-risk MDS fall into the international prognostic scoring system-revised (IPSS-R) categories of very low-, low-, and intermediate-risk groups and those

with higher-risk MDS into the high- and very high-risk groups [20].

### Cytologic analysis

Bone marrow smears from diagnosis were reviewed using an avidin–biotin-complex method (ABC; CD41 immune staining) by the experts who were blinded for patients' diagnoses, cytopenias and cytogenetic status in cytology. The preparation of bone marrow smear was a relatively uniform procedure. The marrow area on every smear was approximate to  $1.5 \times 3.0$  cm with proper and relatively uniform thickness.  $\geq 30$  megakaryocytes were evaluated and the frequency of morphologic abnormalities was recorded. The presence of nuclear hypolobation, single or multiple separate small round nuclei were considered as main characteristics of dys-megakaryocytopenia.

### Statistical analyses

Statistical analyses were performed using SPSS 19.0 software or SAS software. The best discriminator threshold was detected using the minimal P value approach (a method aimed at minimizing the identification of rare classes of subjects) and considering survival (log-rank statistic) as the dependent variable [21, 22]. The functional form of the covariate under study was also evaluated using Martingale residual analysis [23].

Numerical variables were summarized by median and range. Categorical variables were described with count and relative frequency (%) of subjects in each category. Comparison of numerical variables between groups was carried out using a non-parametric approach (Mann–Whitney test). Comparison of the distribution of categorical variables in different groups was performed with  $\chi^2$  test (unordered categorical variable) or the non-parametric approach (ordinal categorical variable).

Median survival was estimated using the Kaplan–Meier method and compared using the log-rank test. Cox proportional hazard regression model was used for multivariate analyses. P values were two-tailed and statistical significance was set as the level of  $P < 0.05$ .

## Results

### Subjects variables

Median age at diagnosis was 50 years (range 16–83 years). 286 subjects (68 %) were male. Distribution of WHO subtypes, IPSS-R cytogenetic category and IPSS-R classification are indicated in Table 1.

### Cyto-morphologic evaluation of megakaryocyte dysplasia

Megakaryocyte dysplasia was detected in 374 subjects (89 %). Median frequency of dysplastic megakaryocytes was 14 % (range 0–100 %). Patients without megakaryocytic dysplasia included RA (17 %), RARS (15 %),

**Table 1 Clinical variables (N = 422)**

	Subjects (n = 422)
Median age (range, years)	50 (16–83)
Male	286 (68 %)
WHO classification	
RA	22 (5 %)
RN	2
RT	3
RARS	24 (6 %)
RCMD	198 (47 %)
RAEB-1	84 (20 %)
RAEB-2	76 (18 %)
MDS-U	8
MDS with del(5q) only	5
IPSS-R cytogenetic category	
Very-good	6
Good	208 (49 %)
Intermediate	143 (34 %)
Poor	23 (5 %)
Very-poor	42 (10 %)
IPSS-R (%)	
Very-low	6
Low	99 (23 %)
Intermediate	135 (32 %)
High	105 (25 %)
Very-high	77 (18 %)

RCMD (42 %), RAEB-1 (17 %), RAEB-2 (6 %), MDS-U (4 %). Dysplastic megakaryocytes were assigned to 7 categories: (1) micro-megakaryocytes (<12  $\mu\text{m}$ ); (2) micro-megakaryocytes (12–40  $\mu\text{m}$ ) with 1 nucleus; (3) micro-megakaryocytes (12–40  $\mu\text{m}$ ) with 2 nuclei; (4) micro-megakaryocytes (12–40  $\mu\text{m}$ ) with multiple (more than 2) nuclei; (5) dysplastic megakaryocytes ( $\geq 40 \mu\text{m}$ ) with 1 nucleus; (6) dysplastic megakaryocytes ( $\geq 40 \mu\text{m}$ ) with 2 nuclei; and (7) dysplastic megakaryocytes ( $\geq 40 \mu\text{m}$ ) with multiple (more than 2) nuclei (Fig. 1). The most frequent dysplastic megakaryocytes were micro-megakaryocytes (12–40  $\mu\text{m}$ ) with 1 nucleus and dysplastic megakaryocytes ( $\geq 40 \mu\text{m}$ ) with 1 nucleus, with median frequency of 28 % (0–91 %) and 29 % (0–78 %), respectively. Distribution of each type is shown in Additional file 1: Figure S1.

To analyze the prognostic impact of dys-megakaryopoiesis, we identified the cutoff point for the two prognostic classes with the greatest differences according to the smallest P value for micro-megakaryocytes at 25 % as well as mono-nucleated dys-megakaryopoiesis at 30 %. Subjects without megakaryocytic dysplasia were all grouped to micro-megakaryocytes <25 % and mono-nucleated dys-megakaryopoiesis <30 %.

### Association between subject variables and megakaryocyte dysplasia

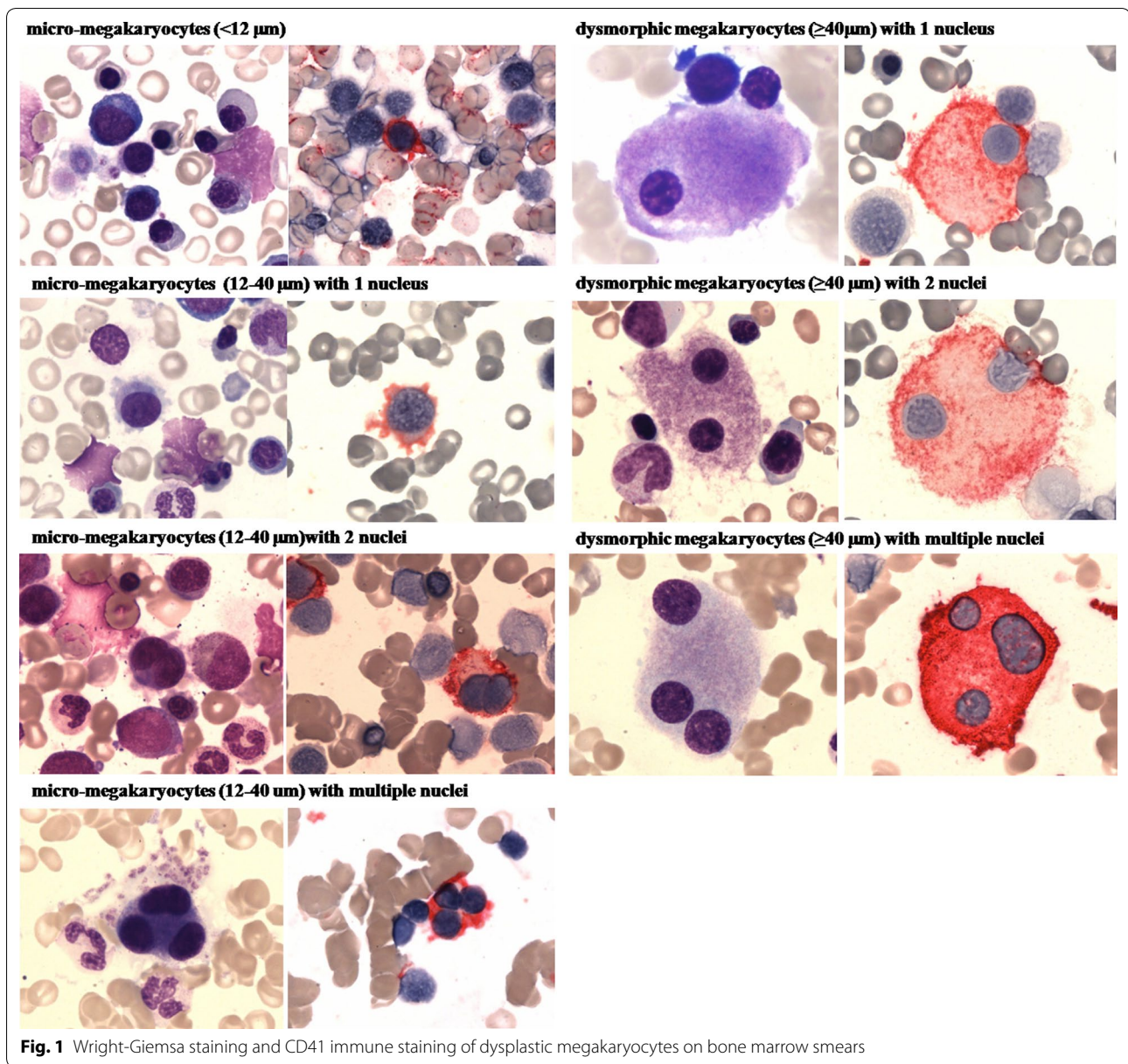
Clinical and laboratory variables in subjects with micro-megakaryocytes <25 % and  $\geq 25$  % are compared in Additional file 1: Table S1. A similar comparison between subjects with mono-nucleated dys-megakaryopoiesis <30 % and  $\geq 30$  % is outlined in Additional file 1: Table S2.

Increased micro-megakaryocytes and mono-nucleated dys-megakaryopoiesis were significantly associated with lower levels of platelet count ( $P < 0.001$  and  $P < 0.001$ ) and higher levels of bone marrow blasts ( $P < 0.001$  and  $P < 0.001$ ). Distributions of WHO 2008 subtypes ( $P = 0.001$  and  $P < 0.001$ ), IPSS-R cytogenetic category ( $P = 0.002$  and  $P = 0.001$ ) and IPSS-R risk cohorts ( $P < 0.001$  and  $P < 0.001$ ) were also significantly different. There was no significant difference in age, gender, hemoglobin concentration and blood neutrophil counts at diagnosis between the two groups. In addition, levels of micro-megakaryocytes and dysplastic mono-nucleated megakaryocytes were significantly associated with abnormal karyotype ( $P = 0.026$  and  $P = 0.014$ ), complex karyotype (CK) ( $P = 0.034$  and  $P = 0.022$ ) and chromosome 7 aberrations ( $P = 0.004$  and  $P = 0.003$ ), but not with monosomal karyotype (MK) or del(5q) (Additional file 1: Table S3).

### Prognostic implications of megakaryocyte dysplasia

In univariate analyses, subjects with micro-megakaryocytes  $\geq 25$  % had poorer survival (median, 19 months [95 % CI 14–23 months]) than those with micro-megakaryocytes <25 % (46 months [28–64 months],  $P < 0.001$ ; Fig. 2a). Similarly, patients with dysplastic mono-nucleated megakaryocytes  $\geq 30$  % demonstrated poorer survival as compared to those with dysplastic mono-nucleated megakaryocytes <30 % (18 months [14–23 months] vs. 49 months [31–68 months],  $P < 0.001$ ; Fig. 2b). Other significant predictors of survival in univariate analyses were male gender ( $P = 0.009$ ), age  $\geq 60$  years ( $P < 0.001$ ), hemoglobin concentration <80 g/L ( $P = 0.001$ ), neutrophils  $< 0.8 \times 10^9 + 9/L$  ( $P = 0.001$ ), platelets  $< 50 \times 10^9 + 9/L$  ( $P = 0.002$ ), bone marrow blasts  $> 10$  % ( $P < 0.001$ ), IPSS-R cytogenetic category ( $P < 0.001$ ) and IPSS-R score ( $P < 0.001$ ).

We performed a multivariate Cox regression model including gender, age, micro-megakaryocytes and IPSS-R. Male gender (hazard ratio [HR] = 1.5; 95 % CI 1.0–2.0;  $P = 0.029$ ), age  $\geq 60$  years (HR = 1.5; [1.1–2.0];  $P = 0.016$ ), micro-megakaryocytes  $\geq 25$  % (HR = 1.6 [1.1–2.2];  $P = 0.010$ ) and IPSS-R score ( $P < 0.001$ ) were significantly associated with survival (Table 2). In a similar analysis including gender, age, dysplastic mono-nucleated megakaryocytes and IPSS-R, only male gender (HR = 1.5; 95 % CI 1.0–2.0;  $P = 0.028$ ), age  $\geq 60$  years

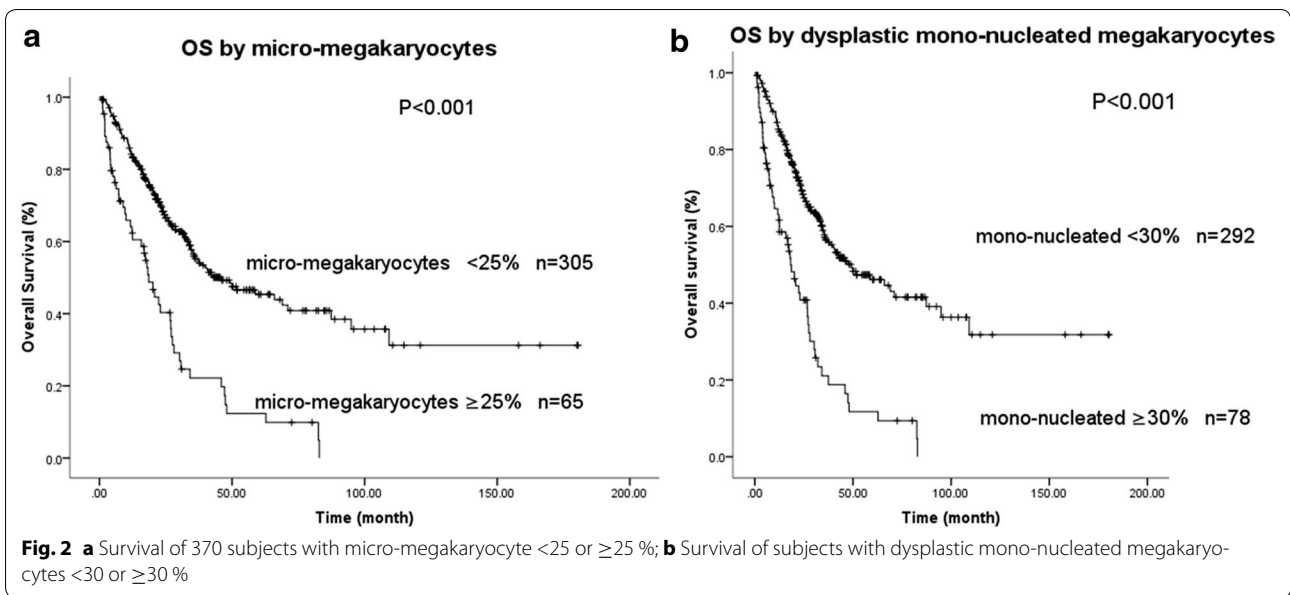


(HR = 1.5; 95 % CI 1.1–2.0;  $P = 0.018$ ), dysplastic mono-nucleated megakaryocytes  $\geq 30$  % (HR = 1.533; [1.1–2.2];  $P = 0.014$ ) and IPSS-R ( $P < 0.001$ ) remained in the final model (Table 3).

#### Prognostic implications of megakaryocytic dysplasia in IPSS-R lower-risk subjects according to the IPSS-R score

In the IPSS-R lower-risk subjects, those with micro-megakaryocytes  $\geq 25$  % had a poorer survival than patients with micro-megakaryocytes  $< 25$  % ( $P < 0.001$ ; Fig. 3a). Similarly, there was a significant difference in survival between patients with mono-nucleated

dys-megakaryopoiesis  $< 30$  and  $\geq 30$  % ( $P < 0.001$ ; Fig. 3b). This association was not significant in subjects in the IPSS-R higher-risk cohort. Consequently, we performed stratified multivariate analyses to further evaluate prognostic implications of megakaryocytic dysplasia in IPSS-R lower-risk subjects only. In the model of cell size including age, gender, IPSS-R and micro-megakaryocytes, only age, IPSS-R and micro-megakaryocytes were significantly correlated with survival (Table 4). In a similar analysis considering dysplastic mono-nucleated megakaryocytes, only age, IPSS-R and dysplastic mono-nucleated megakaryocytes were significantly correlated with survival (Table 5).



**Table 2 Multivariate Cox regression analysis with respect to micro-megakaryocytes**

	HR	95 % CI	P value
Gender			0.029
Male	1.458	1.039–2.047	
Female*	1.0		
Age (year)			0.016
≥60	1.485	1.078–2.047	
<60*	1.0		
IPSS-R			<0.001
Very-low	0	0–2.31E133	
Low	0.106	0.062–0.180	
Intermediate	0.227	0.151–0.341	
High	0.597	0.410–0.869	
Very-high*	1.0		
Micro-megakaryocytes (%)			0.010
≥25	1.575	1.113–2.230	
<25*	1.0	1.0	

\* The reference

**Table 3 Multivariate Cox regression analysis with respect to dysplastic mono-nucleated megakaryocytes**

	HR	95 % CI	P value
Gender			0.028
Male	1.465	1.042–2.058	
Female*	1.0		
Age (year)			0.018
≥60	1.474	1.068–2.034	
<60*	1.0	–	
IPSS-R			<0.001
Very-low	0	0–5.24E132	
Low	0.108	0.063–0.185	
Intermediate	0.235	0.155–0.355	
High	0.607	0.416–0.885	
Very-high*	1.0		
Mono-nucleated (%)			0.014
≥30	1.533	1.089–2.160	
<30*	1.0		

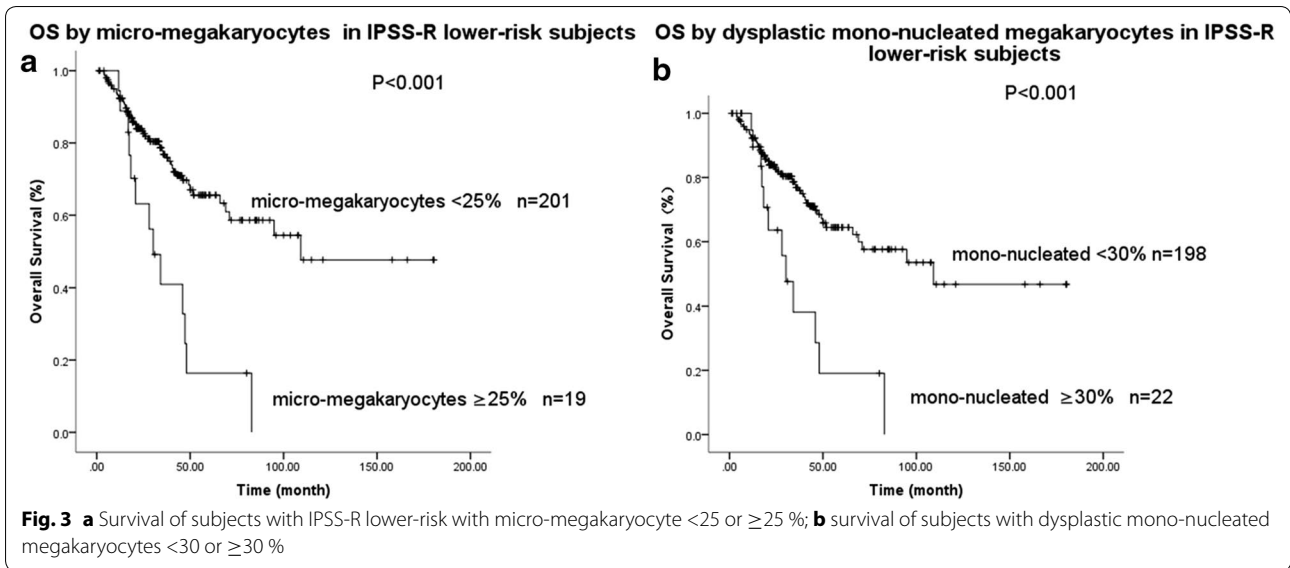
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**Discussion**

In the WHO classification of myeloid neoplasms bone marrow dysplasia ≥10 % of the cells of a specific myeloid lineage is the cardinal diagnostic feature of the MDS. However, morphology is a subjective parameter under the routine staining conditions. For example, concordance for dys-megakaryopoiesis amongst observers was less than concordance for dys-granulopoiesis and dys-erythropoiesis [4]. Thus, specific immune staining may be a better way to evaluate megakaryocytes than routine histological methods [24].

Based on data from our study we propose a systematic classification of dys megakaryopoiesis on bone marrow films of persons with suspected MDS using CD41 immune staining. A study has reviewed marrow smears of 26 RA and 28 RAEB patients, and micro-megakaryocytes were identified in 39.3 and 46.5 % cases under Wright-Giemsa staining, compared with 92.3 and 100 % under CD41 immune staining ( $\chi^2$  test:  $P < 0.005$  and  $P = 0.01$ ) (Wenyu Cai, unpublished data).

In our study, a larger number of dysplastic megakaryocytes was significantly associated with decreased platelet



**Table 4** Multivariate analysis of the survival of IPSS-R lower-risk subjects with respect to micro-megakaryocytes

	HR	95 % CI	P value
Age (year)			0.024
$\geq 60$	1.856	1.086–3.171	
$< 60^*$	1.0		
Gender			0.185
IPSS-R			0.026
Very-low	0	0–6.17E272	
Low	0.478	0.280–0.816	
Intermediate*	1.0		
Micro-megakaryocytes (%)			$< 0.01$
$\geq 25$	2.806	1.509–5.218	
$< 25^*$	1.0		

\* The reference

count, increased bone marrow blasts and increased IPSS-R scores. Cytogenetic abnormalities are associated with characteristic dysplastic features, e.g. isolated del(5q) and hypo-lobed and un-lobed megakaryocyte nuclei and del(17p) with hypo-lobed neutrophil nuclei [25]. Data from our study also indicated parallel increases in micro-megakaryocytes and dysplastic mono-nucleated megakaryocytes and IPSS-R cytogenetic scores, as well as frequencies of abnormal karyotype, CK and chromosome 7 aberrations. However, according to our study, there was no significant association of dysplastic megakaryocytes with del(5q). Hypolobulation is also commonly seen in all other subtypes besides MDS with del(5q) only. This unexpected result may be on account of the small proportion of del(5q) patients in our study cohort (5/422). A larger

**Table 5** Multivariate analysis of the survival of IPSS-R lower-risk subjects with respect to dysplastic mono-nucleated megakaryocytes

	HR	95 % CI	P value
Age (year)			0.03
$\geq 60$	1.808	1.058–3.089	
$< 60^*$	1.0		
Gender			0.161
IPSS-R			0.018
Very-low	0	0–1.07E266	
Low	0.463	0.272–0.789	
Intermediate*	1.0		
Mono-nucleated (%)			$< 0.01$
$\geq 30$	2.672	1.410–5.064	
$< 30^*$	1.0		

\* The reference

dataset is necessary for further research on this issue. Furthermore, we suspect that abnormalities in process of endo-reduplication may play a more important role than aberrant endomitosis in dysplastic megakaryocytes, for micro-megakaryocytes with 1 nucleus and dysplastic megakaryocytes ( $\geq 40 \mu\text{m}$ ) with 1 nucleus were most frequently seen in our study.

Studies reported a threshold of 10 % dysplastic granulocytes as well as 30–40 % dysplastic megakaryocytes as the best survival discriminator [4, 11, 26, 27]. We found a threshold of 25 % of micro-megakaryocytes and 30 % of dysplastic mono-nucleated megakaryocytes as the best survival discriminators, which was independent of age, gender and IPSS-R risk score. We also found

micro-megakaryocytes and dysplastic mono-nucleated megakaryocytes were independently associated with survival in subjects with IPSS-R lower-risk MDS. Based on these data we suggest that adding these variables to the IPSS-R model could improve the predictive accuracy in untreated subjects and those with IPSS-R lower-risk MDS. External validation of concordance between observers using our technique and dysplastic megakaryocytes classification is needed.

## Additional file

**Additional file 1.** Distribution of dysplastic megakaryocytes in subjects and variables of subjects with dysplastic megakaryocytes.

## Authors' contributions

ZJX designed the research, was the principal investigator, and took primary responsibility for the paper; GF acquisition of data, analysis and interpretation of data, and drafting the article; WC, WC, GH acquisition of data; TJQ, YZ, LWF, HLZ, LJP, NBH, SQQ, BL and ZJX recruited the patients; ZJX, RPG, JMB, PLG wrote the typescript. All authors read and approved the final manuscript.

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## Competing interests

RPG is a part-time employee of Celgene Corp. The authors declare that they have no competing interests.

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