The non-erythroid myeloblast count rule in myelodysplastic syndromes: fruitful or futile?

One concept may have multiple terms, and one term can harbor multiple concepts. The concept of erythroid cells undergoing proliferation analogous to granulocytes in leukemia has been described as erythroleukemia, erythremic myelosis, Di Guglielmo syndrome, M6 acute myeloid leukemia (AML), acute erythroleukemia (AEL), and pure erythroid leukemia.[1-5] Additionally, erythroid predominance has been associated with various disorders including benign conditions and myelodysplastic syndromes (MDS). In MDS, the issue of erythroid predominance is subject to debate as the clinical approach and underlying pathophysiology are questionable. Although the 2016 revision of the World Health Organization (WHO) eliminated the non-erythroid blast count (NEBC) rule that advised enumerating marrow myeloblast percentages from non-erythroid cells in MDS with erythroid predominance (MDS-E), studies in favor and against this rule are still being published (*Online Supplementary Table S1*).[6-9] To study the published contradictions regarding the NEBC rule, we retrospectively investigated a cohort of 280 patients from our institutional registry classified into appropriate subgroups by marrow erythroid cell-

Table 1. Observations on the use of the non-erythroid blast count rule and the clinicopathological characterization of myelodysplastic syndromes with erythroid predominance from selected publications as compared to our study.

		Wang	Arenillas	Calvo	Bennett	Spronsen
Use of the NEBC rule	**WHO classification in MDS-E**					
	NEBC rule creates RAEB-2 category in MDS-E - pro	√				
	NEBC rule upgraded MDS-E have lower OS - pro		√			
	NEBC rule upgraded MDS-E have equal LFS and OS time - con				√	√
	No improvement of prognostic weight - con				√	√
	IPSS-R in MDS-E					
	NEBC rule creates high risk category in MDS-E - pro		√			
	NEBC rule upgraded MDS-E have lower OS - pro		√			
	NEBC rule upgraded very low- to intermediate risk MDS-E have lower OS - pro			√		
	Improvement of prognostic weight - pro			√	√	
	NEBC rule upgraded MDS-E have equal LFS/OS - con				√	√
	No improvement of prognostic weight - con				√	√
	IPSS-R in MDS in general					
	NEBC rule upgraded very low- to intermediate-risk MDS have lower OS - pro			√		
	NEBC rule upgraded intermediate-risk MDS have higher leukemia-related death - pro			√		
	Improvement of prognostic weight - pro				√	
	No improvement of prognostic weight - con					√
MDS-E as separate entity	**Clinical outcome**					
	MDS-E have comparable OS as MDS-NE despite lower myeloblast percentages	√	√			
	MDS-E have comparable LFS and OS times as MDS-NE				√	
	MDS-E have longer OS and LFS times than MDS-NE					√
	Karyotype					
	MDS-E have higher frequency of IPSS cytogenetic intermediate- plus high risk	√				
	MDS-E and MDS-NE have similar cytogenetic risks		√			
	MDS-E have higher frequency of del(20q)					√
	Morphology					
	MDS-E have profound dyserythropoiesis			√		
	No differences between MDS-E and MDS-NE					√
	Peripheral blood counts					
	MDS-E have less severe thrombocytopenia		√			
	No differences between MDS-E and MDS-NE	√				√
	Age at time of diagnosis					
	MDS-E have a younger age at time of diagnosis	√	√			
	No differences between MDS-E and MDS-NE					√

NEBC: non-erythroid blast count; WHO: World Health Organization; MDS: myelodysplastic syndromes; MDS-E: MDS with erythroid predominance; MDS-NE: MDS with non-erythroid predominance; RAEB-2: refractory anemia with excess blasts type 2; OS: overall survival; LFS: leukemia-free survival; IPSS: International Prognostic Scoring System; IPSS-R: revised IPSS.

and myeloblast percentages following the WHO 2008 and 2016 criteria (Figure 1A). We performed survival analysis with censoring of patients undergoing stem cell transplantation or induction chemotherapy, evaluated the performance of the clinical risk scores with and without applying the NEBC rule using Harrell's concordance index C, and questioned current definition of erythroid predominance. Our data show that MDS-E comprise both indolent and aggressive subtypes and that erythroid predominance can be a transient condition. We conclude that the NEBC rule is of no value based on its prognostic irrelevance and the inter- and intra-patient variety in MDS-E. Instead, we suggest refining the current definition of erythroid predominance: a relative increase in erythroid cells of at least 50% of total marrow cells.

On observing that MDS-E have a comparable outcome as MDS-NE despite lower myeloblast percentages, investigators stated that MDS-E patients have a poor prognosis that is inadequately recognized by myeloblast percentages from total marrow cells.[6,8] Still, one may question whether MDS-E behave more aggressively than MDS-NE and as predicted by myeloblast percentages. In

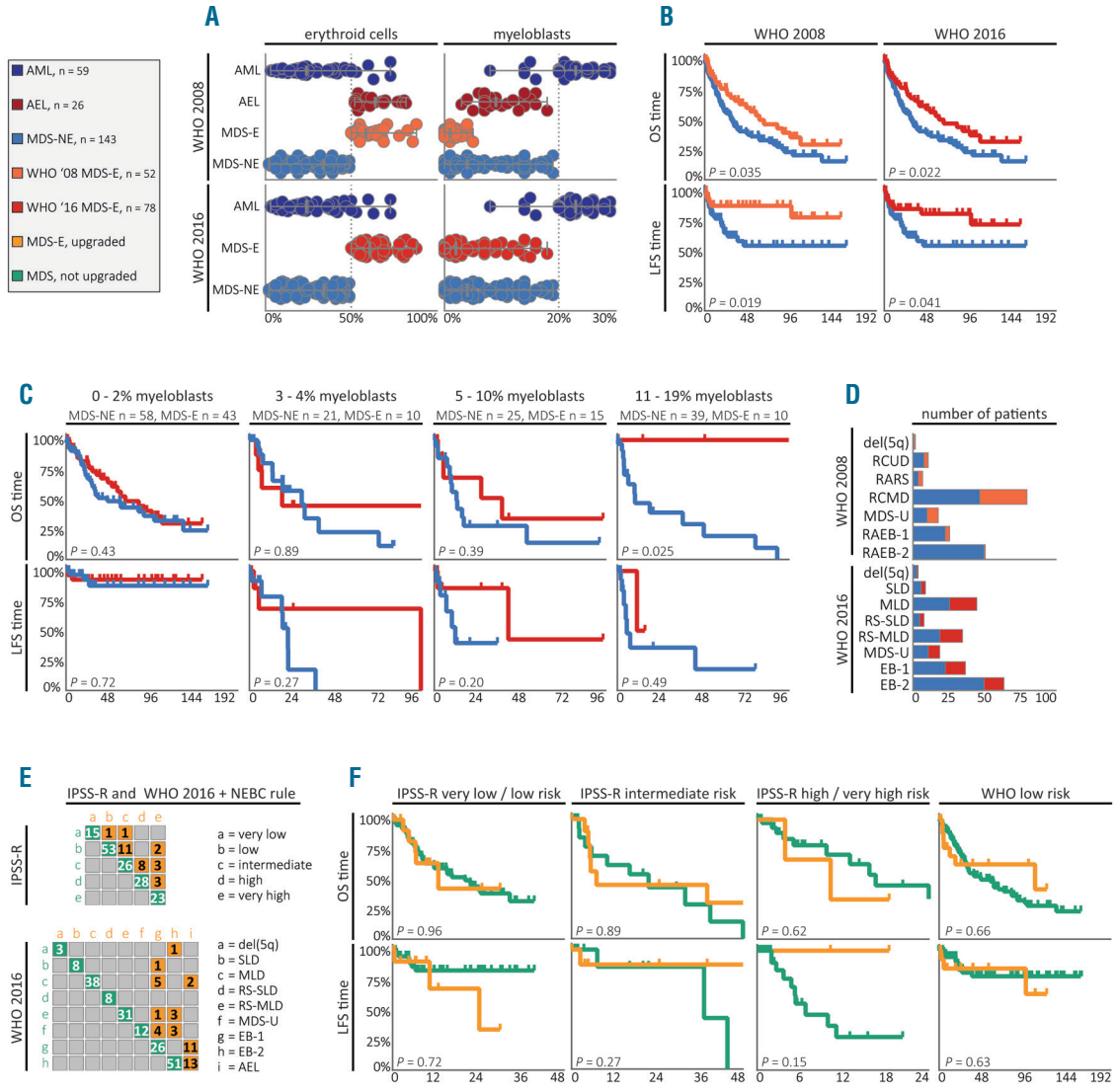

Figure 1. Study on the value of the non-erythroid blast count (NEBC) rule. All Kaplan-Meier estimates illustrate the overall survival (OS) and leukemia-free survival (LFS) times in months. (A) Marrow erythroid cell- and myeloblast percentages from total bone marrow cells distributed by diagnosis according to the World Health Organization (WHO) 2008 and 2016 criteria. (B) MDS-E patients myelodysplastic syndromes with erythroid predominance show an improved clinical outcome as compared to MDS-NE patients in spite of the used WHO criteria. (C) WHO 2016 and MDS-NE patients show a comparable clinical outcome when stratifying by marrow myeloblast percentages, except for MDS patients with ≥11% myeloblasts in spite of the small sample size. (D) The WHO 2008 and 2016 classify less MDS-E than MDS-NE patients as refractory anemia with excess blasts type 2 (RAEB-2) and excess blasts type 2 (EB-2). (E) Impact of the use of the NEBC rule within the revised International Prognostic Scoring System (IPSS-R) and WHO 2016 classification. The number refers to the absolute amount of patients classified according to the IPSS-R and WHO 2016 classification without use of the NEBC rule (in green) and with use of the NEBC rule (in orange). (F) Clinical outcome of MDS-E patients initially classified as IPSS-R very low- or low-, IPSS-R intermediate-, IPSS-R high- or very high- and WHO low-risk who are upgraded to a higher risk category by the NEBC rule in comparison with MDS-E and MDS-NE patients who remained classified into initial categories. WHO low-risk is defined as <2% and <5% peripheral blood- and bone marrow myeloblast percentages, respectively. del(5q): MDS with isolated del (5q); RCUD: refractory cytopenia with unilineage dysplasia; RARS: refractory anemia with ring sideroblasts; RCMD: refractory cytopenia with multilineage dysplasia; MDS-U: MDS unclassifiable; RAEB-1: refractory anemia with excess blasts type 1; SLD: single lineage dysplasia with ring sideroblasts; MLD: multilineage dysplasia; RS-SLD: single lineage dysplasia with ring sideroblasts; RS-MLD: multilineage dysplasia with ring sideroblasts; EB-1: excess blasts type 1; EB-2: excess blasts type 2.

our study cohort of 280 patients (see details in *Online Supplementary Appendix*), MDS-E patients have a longer leukemia-free survival (LFS) and overall survival (OS) time than MDS-NE, irrespective of which WHO criteria are used (Figure 1B). Whereas the median percentage of marrow myeloblasts is lower in MDS-E than MDS-NE (WHO 2008: 1% *vs.* 4%, $P<0.001$; WHO 2016: 2% *vs.* 4%, $P=0.013$), these percentages have prognostic value for overall survival (OS) and leukemia-free survival (LFS) in MDS-E and MDS-NE (*Online Supplementary Table S2*). To study whether erythroid predominance leads to a misinterpretation of myeloblast percentages such that the prognosis of MDS-E is underestimated, we compared the outcome of WHO 2016 MDS-E and MDS-NE patients stratified by marrow myeloblast percentages. Interestingly, MDS-E and MDS-NE with corresponding myeloblast percentages show comparable outcomes, except for MDS-E with >10% myeloblasts who have a longer OS time (median not reached, $P=0.025$) (Figure 1C). In spite of our small sample size, these observations challenge the presumption that marrow myeloblast percentages lose their prognostic value in the presence of erythroid predominance. Rather, MDS-E may represent an indolent disorder that is accurately captured by low myeloblast percentages.

If MDS-E do not behave *per se* aggressively, only improved risk stratification would justify the continued use of the NEBC rule. However, the NEBC rule does neither increase the performance for predicting LFS and OS times of marrow myeloblast percentages nor of the IPSS-R and WHO criteria (*Online Supplementary Table S2*). Still, we elaborate on the impact of the NEBC rule on risk stratification for sake of comparison with other studies (Table 1). When enumerating myeloblast percentages from total marrow cells, risk distribution by the IPSS-R was comparable between MDS-E and MDS-NE (*Online Supplementary Table S3A*). In contrast, the WHO 2008 and 2016 criteria reflect the favorable prognosis of MDS-E, as fewer MDS-E than MDS-NE patients are classified as refractory anemia with excess blasts type 2 (6% *vs.* 16%, $P<0.001$) and, despite inclusion of AEL, excess blasts type 2 (8% *vs.* 35%, $P<0.001$), respectively (Figure 1D). When using the NEBC rule, a proportion of the MDS-E patients are upgraded within the IPSS-R and WHO criteria, respectively (Figure 1E). We found no difference between the outcome of upgraded MDS-E patients and MDS-E and MDS-NE patients remaining classified within initial categories (Figure 1F). These data contradict the presumption that the NEBC rule identifies an unfavorable MDS-E subgroup within distinct risk categories. Interestingly, Calvo *et al.* recommend the use of the NEBC rule for better risk stratification of MDS in general.[9] Note that this recommendation is based on a marginally increased concordance probability estimate as a reflection of performance of the IPSS-R for predicting LFS and OS time. We observe an increase and decrease in concordance of 2% for predicting LFS and OS time, respectively, when using the NEBC rule in all MDS patients (*Online Supplementary Table S2*). Accordingly, our data do not support the use of the NEBC rule in any of the MDS patients. Although myeloid neoplasms with erythroid predominance are generally typified by poor karyotypes, multilineage dysplasia, pancytopenia and increased cellularity, the WHO does no longer define AEL separately.

Questioning whether AEL and MDS-E should be

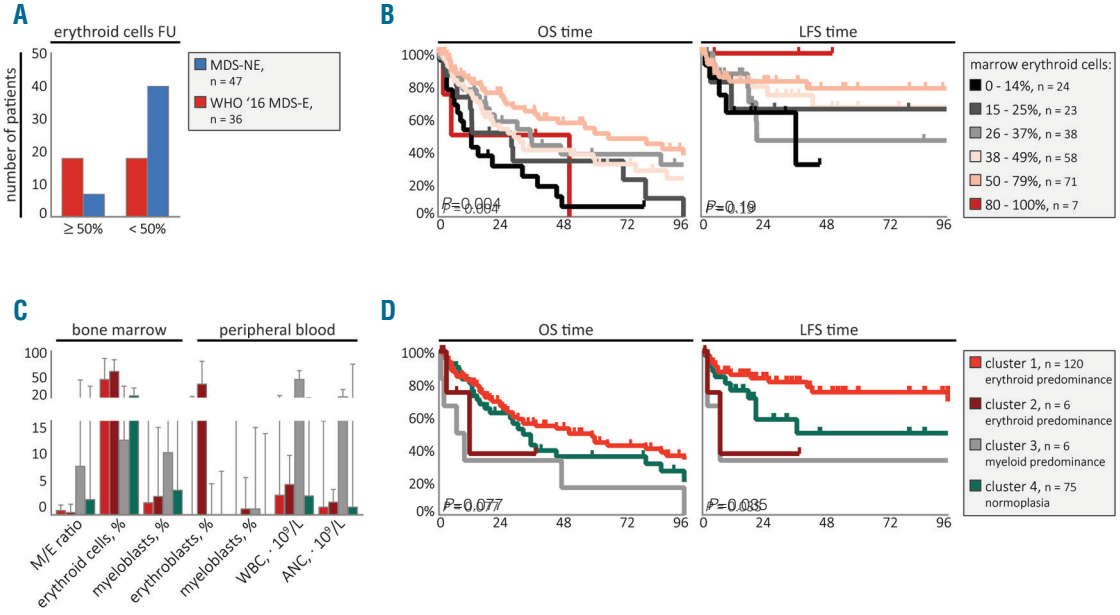

Figure 2. Study on the nature of erythroid predominance. (A) The absence or presence of erythroid predominance defined as ≥ 50% marrow erythroid cells at median follow-up time of 35 (1-119) and 16 (1-119) months in World Health Organization (WHO) 2016 myelodysplastic syndromes (MDS) with erythroid predominance (MDS-E) and MDS with non-erythroid predominance (MDS-NE) patients, respectively. Half of the MDS-E patients have either normalization or persistence of erythroid predominance. Most MDS-NE patients maintain a non-erythroid predominant bone marrow. (B) The prognostic value of distinct marrow erythroid cell percentages. MDS patients with less than 15% or at least 80% marrow erythroid cells have the poorest clinical outcome. (C) K-means clustering based on bone marrow and peripheral blood cell counts was applied to identify patient clusters. Note that K-means clustering was based on all these features except for the percentage of peripheral blood myeloblasts. (D) Clinical outcome of WHO 2016 MDS patients stratified by K-means clustering. Cluster 2 and 3, the aggressive erythroid predominance and myeloid predominance subtype, respectively, show the poorest clinical outcome. All Kaplan-Meier estimates illustrate the overall survival (OS) and leukemia-free survival (LFS) times in months.

defined as unique entities, we searched for distinctive clinicopathological features across available publications (Table 1) and our patients. Despite poor peripheral blood counts (*Online Supplementary Table S3B*), AEL patients have a longer OS than MDS patients with excess blasts (median not reached *vs.* 8 months, *P*=0.005) and AML patients (median not reached *vs.* 13 months, *P*=0.046) (*data not shown*). This suggests that AEL patients do not have a poor prognosis that justifies promotion to AML, supporting its inclusion within the MDS spectrum. Compared to MDS-NE, our MDS-E patients had an increased incidence of the low-risk cytogenetic abnormality del(20q) and higher proportions of ring sideroblasts, suggestive for SF3B1 as underlying mechanisms (*Online Supplementary Table S3B*). The favorable prognostic profile of MDS-E is in contrast with the literature, which may indicate the heterogeneity underlying erythroid predominance possibly due to the non-specificity of a relative increase in marrow erythroid cells.

The last question that should be asked is whether a relative threshold of 50% marrow erythroid cells sufficiently defines erythroid predominance. First, we studied the kinetics of 50% marrow erythroid cells using repeated marrow aspirations of patients who were not treated with induction chemotherapy or autologous or allogeneic transplanted. Whereas an equal number of MDS-E patients have normalization or persistence of erythroid predominance at the time of follow up, most MDS-NE patients maintain a non-erythroid predominant marrow (Figure 2A). This suggests that erythroid predominance can be a transient condition that may be restored naturally, whereas its onset during the disease is uncommon. Second, we investigated the prognostic value of marrow erythroid cell percentages following Bennett *et al*. In contrast to the 50% threshold, we observed that extremely low and high marrow erythroid cell percentages have prognostic value: MDS with ≤15% marrow erythroid cells and MDS-E with ≥80% marrow erythroid cells have a poorer outcome than other MDS (OS: 16 *vs.* 47 months, *P*=0.006; LFS: median not reached, *P*=0.049) and MDS-E with <80% marrow erythroid cells (OS: 5 *vs.* 38 months, *P*=0.024), respectively (Figure 2B). Finally, with the aim of differentiating between erythroid predominance and myeloid hypoplasia, we related myeloid/erythroid (M/E) ratios to marrow- and peripheral blood cell counts using K-means clustering. Based on statistically significant M/E ratios, marrow myeloblast and erythroid cell percentages, blood erythroblast percentages and white blood cell and neutrophil counts, we identified four clusters of which two represented erythroid predominance (Figure 2C and *Online Supplementary Table S4*). Survival analysis suggests that erythroid predominance clusters comprised an indolent and aggressive subtype. Patients with an aggressive subtype had a lower age at the time of diagnosis (*P*=0.012) and a higher percentage of circulating erythroblasts (*P*=0.024) than patients with an indolent subtype (Figure 2D). These explorative analyses reveal the heterogeneity underlying MDS-E and suggest parameters for refining the current definition of erythroid predominance.

Based on this study, we conclude that there is no reason to use the NEBC rule. First, MDS-E are not a uniformly aggressive entity but are accurately diagnosed by myeloblast percentages from total bone marrow cells. Second, the NEBC rule does not improve risk stratification in either MDS-E or MDS in general. To prevent illegitimately upgrading MDS patients with indolent erythroid predominance, we support the decision of the WHO to reject the NEBC rule from clinical practice. We realize that this study is limited by its retrospective nature, sample size and referral bias, with patients selected from our tertiary-care center. However, we question whether the effect of such bias will be to underestimate the value of the NEBC rule. We expect that differences in reasoning and methodology might explain the controversy between our conclusions and previous studies.[6,8,9] In the future, we wish not only for prospective population-based studies to achieve an evidence-based approach towards MDS-E, but also for functional and genomic studies to connect clinical heterogeneity of erythroid predominance and the underlying pathophysiology.[10-12]

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References

1. Dameshek W, Baldini M. The Di Guglielmo syndrome. Blood. 1958; 13(2):192-194.
2. Dameshek W. The DiGuglielmo syndrome revisited. Blood. 1969; 34(5):567-572.
3. Bain BJ. Di Guglielmo and his syndromes. Br J Haematol. 2003; 120(6):939-943.
4. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-2405.
5. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009;114(5):937-951.
6. Wang SA, Tang G, Fadare O, et al. Erythroid-predominant myelodysplastic syndromes: enumeration of blasts from nonerythroid rather than total marrow cells provides superior risk stratification. Mod Pathol. 2008;21(11):1394-1402.
7. Bennett JM, Tuechler H, Aul C, Strupp C, Germing U. Dysplastic erythroid precursors in the myelodysplastic syndromes and the acute myeloid leukemias: Is there biologic significance? (How should blasts be counted?). Leuk Res. 2016;47:63-69.
8. Arenillas L, Calvo X, Luno E, et al. Considering Bone Marrow Blasts From Nonerythroid Cellularity Improves the Prognostic Evaluation of Myelodysplastic Syndromes. J Clin Oncol. 2016;34(27):3284-3292.
9. Calvo X, Arenillas L, Luno E, et al. Enumerating bone marrow blasts from nonerythroid cellularity improves outcome prediction in myelodysplastic syndromes and permits a better definition of the intermediate risk category of the Revised International Prognostic Scoring System (IPSS-R). Am J Hematol. 2017;92(7):614-621.
10. Park S, Picard F, Dreyfus F. Erythroleukemia: a need for a new definition. Leukemia. 2002;16(8):1399-1401.
11. Grossmann V, Bacher U, Haferlach C, et al. Acute erythroid leukemia (AEL) can be separated into distinct prognostic subsets based on cytogenetic and molecular genetic characteristics. Leukemia. 2013; 27(9):1940-1943.
12. Cervera N, Carbuccia N, Garnier S, et al. Molecular characterization of acute erythroid leukemia (M6-AML) using targeted next-generation sequencing. Leukemia. 2016;30(4):966-970.