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

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Letter to the Editor

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Supplementary File

Methods

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Literature study

A literature study was performed to list all publications in favor or against the NEBC rule in MDS. Fifty-nine studies (search until 11th of July 2018) were identified of which 4 studies were selected based on information reported in the abstract (**Table S1**). The advanced searching option in the PubMed database was used with "myelodysplastic syndromes" as a medical subject heading (MeSH) combined with one of the following title/abstract (TiAb) features: "erythroid precursors", "erythroid proliferation", "erythroid hyperplasia", "erythroid-predominance" or "erythroid-predominant". The search was restricted to research with available abstracts studying human adults above 18 years of age and written in the English language over the last ten years. Case reports and reviews were excluded, whereas clinical studies comparing methods of myeloblast enumeration at the time of diagnosis were included. Outcomes of interest were the prognostic assessment and the clinicopathological characterization of MDS with erythroid hyperplasia.

Patient groups

The MDS/AML registry at the Amsterdam UMC, location Vrije Universiteit Amsterdam comprises 363 patients who have been diagnosed with MDS according to the FAB or WHO classifications between 2000 and 2012. Patients with incomplete data on bone marrow smears or follow-up and AML patients with ≥ 30% myeloblasts of total marrow cells were excluded from the analysis (n = 83). Included MDS and AML patients (n = 280) were reclassified according to the WHO 2008 and 2016 classifications with myeloblast enumeration from total nucleated cells and non-erythroid cells.[1,2] Patient diagnosed with MDS were assigned as MDS with erythroid proliferations (MDS-E) when nucleated erythroid cells constituted ≥50% of total marrow cells. The final analysis included 52 (19%) MDS-E, 143 (51%) MDS-NE, 26 (9%) AEL and 59 (21%) AML patients and 78 (28%) MDS-E, 143 (51%) MDS-NE and 59 (21%) AML patients following the WHO 2008 and 2016 criteria, respectively. Seven patients had at least 80% nucleated erythroid cells of total marrow cells but less than 30% proerythroblasts. Therefore, these patients did not fulfill the diagnostic criteria for PEL and were not excluded from this study. [2] Patients with secondary MDS (n = 19, 7%) were equally distributed amongst different diagnoses and therefore not excluded. The percentage of marrow myeloblasts and erythroblasts in MDS-E and patient control groups are shown in **Figure S1**. This study was approved in accordance with the Helsinki Declaration by the Medical Ethics Committee of the Amsterdam UMC, location Vrije Universiteit Amsterdam.

Data collection

Erythroid hyperplasia was defined as the presence of at least 50% nucleated erythroid cells of total marrow cells. For comparison with published data, plasma cells and lymphocytes were not excluded from total marrow nucleated count. Besides erythroid percentages of total marrow nucleated count, myeloid/erythroid (M/E) ratios were calculated using the formula: myeloblasts + (pro-/meta)myelocytes + neutrophils + eosinophils + basophils + (pro)monocytes) / nucleated erythroid cells. Based on expert opinion, we defined M/E ratios ranging between 1.2:1 and 5:1 as normal. The IPSS-R could be applied for risk stratification in 64 (82%) and 110 (77%) of the WHO 2016 MDS-E and MDS-NE patients. [3] The WHO classifications and IPSS-R were applied with and without application of the NEBC rule in MDS-E and MDS in general. Following the WHO criteria, thresholds of 10% abnormal cells in a specific hematopoietic lineage, and hemoglobin levels below 10 g/dL, platelet counts below $100 \cdot 10^9$/L, and neutrophil counts below $1.8 \cdot 10^9$/L were adopted for defining dysplasia and cytopenias, respectively. Since screening for SF3B1 mutations has not been performed in this cohort, a cut-off of at least 15% ring sideroblasts of erythroid precursors was applied for defining their presence. Bone marrow biopsies were carried out in 233 (83%) of the patients. Following the European Myelofibrosis Network recommendations, marrow fibrosis was graded

into the categories no, mild, moderate, and severe. [4] Bone marrow cellularity was evaluated in the context of age. Cytogenetic analysis was performed in 238 (85%) of the patients using conventional karyotyping or fluorescence in situ hybridization when insufficient metaphases could be analyzed. Cytogenetic findings were documented following the International System for Human Cytogenetic Nomenclature. [5] Medical records were reviewed to collect information on patients' medical history, treatment, and disease course. If necessary, primary care physicians were contacted to get information on the disease course. Patients were followed for survival through January 2015 (median: 23 (0 - 177) months).

Statistical analysis

Categorical data were given as frequencies with percentages. All continuous data followed a non-normal distribution and were given as median with range. Mann-Whitney U tests and Kruskall-Wallis tests were applied for testing numerical data between 2 groups or between ≥ 3 groups, respectively. Chi-square tests were applied for testing categorical data in contingency tables. K-means clustering was used to explore whether MDS would be grouped in different clusters based on M/E ratios and blood and marrow cell counts. Survival curves were constructed using the Kaplan-Meier method and tested significantly by the log-rank test. The leukemia-free survival (LFS) and overall survival (OS) time were defined as the number of months from the date of diagnosis until date of leukemic transformation and date of death or last follow up, respectively. The follow-up survival time was defined as the number of months from the date of the repeated bone marrow aspiration until the date of death or last follow up. Patients undergoing stem cell transplantation or induction chemotherapy were censored at the date of start treatment. Patients receiving supportive care, lenalidomide or azacitidine were not censored. The performance of the WHO classifications and revised International Prognostic Scoring System (IPSS-R) was evaluated using Harrell's concordance index *C.* The *C*-statistic estimates the concordance between predicted probabilities and observed outcomes. A value of 0.5 indicates random predictions, whereas 1 means perfect predictions wherein all pairs are concordant. Confidence intervals with 95% coverage were used and two-sided *P*-values < .05 were considered statistically significant. Analyses were conducted with the Statistical Package for the Social Sciences version 22 and the statistical software R 3.4.2 using the packages "survival" (Therneau).

References

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Study	**Wang et al. 2008**	**Bennett et al. 2016**	**Arenillas et al. 2016**	**Calvo et al. 2017**	**Spronsen et al. 2019**
Opinion on NEBC rule	pro	con	pro	pro	con
MDS cohort					
n	266	1448	3692 (GESMD)	3924 (GESMD)	221
MDS-E / MDS-NE	74 / 192	unknown	465 / 3227	498 / 3426	78/143
Cytotoxic therapy	unknown	no	unknown	yes	yes
Risk stratification					
Disease classification	WHO 2001	WHO 2008	WHO 2008	WHO 2008	WHO 2008* WHO 2016
Risk stratification	IPSS	IPSS-R	IPSS	IPSS-R	IPSS-R
Statistical methodology					
Non-parametric tests	yes	unknown	yes	unknown	yes
Prognostic power analysis	no	*Dxy* index	*C* index	*C* index	*C* index
Approach to treated cases in survival analysis	unknown	no censoring	censoring start therapy	censoring start therapy	censoring start therapy

Table S1. Available publications on the NEBC rule in MDS differ in conclusion and methodology. *Difference between the WHO 2008 and 2016 is that the WHO 2016 includes patients classified as AEL within the WHO 2008. Abbreviations: GESMD, Spanish Group of Myelodysplastic Syndromes.

	- NEBC rule		+ NEBC rule	
	OS time	**LFS time**	**OS time**	**LFS time**
Marrow myeloblasts				
WHO 2016 MDS-NE	*P* = .013	*P* < .001		
	C = .65	*C* = .78		
WHO 2016 MDS-E	*P* = .002	*P* = .031	*P* = .016	*P* = .18
	C = .41	*C* = .72	*C* = .39	*C* = .70
WHO 2008 classification*				
MDS-NE	*P* = .075	*P* = .002		
	C = .61	*C* = .75		
MDS-E	*P* = .048	*P* = .21	*P* = .076	*P* = .68
	C = .63	*C* = .72	*C* = .63	*C* = .68
WHO 2016 classification*				
MDS-NE	*P* = .049	NA		
	C = .60	*C* = .74		
MDS-E	*P* = .081	*P* = .38	*P* = .11	*P* = .57
	C = .60	*C* = .72	*C* = .59	*C* = .68
IPSS-R				
WHO 2016 MDS-NE	*P* < .001	*P* < .001		
	C = .67	*C* = .77		
WHO 2016 MDS-E	*P* = .065	*P* = .10	*P* = .050	*P* = .23
	C = .66	*C* = .70	*C* = .70	*C* = .73
WHO 2016 MDS in general	*P* < .001	*P* < .001	*P* < .001	*P* < .001
	C = .67	*C* = .75	*C* = .65	*C* = .77

Table S2: The NEBC rule does not improve the prognostic performance of marrow myeloblast percentages, the WHO 2008 and 2016 classifications and IPSS-R in MDS-E and MDS-NE. *Difference between the WHO 2008 and 2016 is that the WHO 2016 includes patients classified as AEL within the WHO 2008. Abbreviations: NA, not applicable.

	MDS-E (n = 52) 2008 WHO	MDS-E (n = 78) 2016 WHO	MDS-NE (n = 143) 2008/2016 WHO	*P* value[1] 2008 WHO	*P* value[2] 2016 WHO
Clinical characteristics					
Age, years	65 (22 - 84)	65 (22 - 84)	65 (32 - 88)	.93	.42
Female/male, %	38.5/61.5[3]	38.5/61.5[3]	36/64	.79	.76
Medical history[4], %					
Hematological malignancy	4	4	3	.72	.68
Solid malignancy	17	12	20	.69	.12
Auto-immune disorder	12	9	16	.48	.12
Cardiovascular disease	33	32	36	.72	.61
Diabetes mellitus	17	15	12	.34	.49
Peripheral blood findings					
Hemoglobin level, g/dL	9.3 (5.6 - 12.9)	9.5 (5.6 - 14.7)	9.8 (5.6 - 15.1)	.11	.35
Neutrophil count, $\cdot 10^9$	2.6 (0 - 6.6)	1.3 (0 - 6.6)	1.5 (0 - 34)	.085	.51
Platelet count, $\cdot 10^9$	143 (1 - 513)	82 (1 - 513)	85 (1 - 729)	.073	.73
Pancytopenia, %	15	26	23	.28	.57
Myeloblasts, %	0 (0 - 9)	0 (0 - 17)	0 (0 - 15)	.024	.16
Erythroblasts, %	0 (0 - 40)	0 (0 - 83)	0 (0 - 69)	.30	.091
Bone marrow morphology, %					
Dyserythropoiesis	96	96	88	.099	.050
Dysgranulopoiesis	31	31	32	.97	.88
Dysmegakaryopoiesis	80	84	86	.36	.66
Multilineage dysplasia	83	82	73	.18	.16
Ring sideroblasts	45 (0 - 95)	41 (0 - 98)	13 (0 - 91)	.001	.009
Myeloblasts, %	1 (0 - 5)	2 (0 - 18)	4 (0 - 19)	<.001	.020
Marrow erythroid cells, %	59 (50 - 88)	61 (50 - 88)	34 (2 - 49)	<.001	<.001
Histopathology, %					
Fibrosis[5]	32	31	37	.53	.40
Hypercellularity	60	54	46	.11	.25
Hypocellularity	2	9	16	.01	.15
Karyotype, %					
Normal karyotype	57	56	57	.94	.89
Complex karyotype	11	11	13	.71	.80
Monosomal karyotype	9	11	9	.86	.66
Deletion 20	10	7	1	<.01	.01
IPSS-R stratification[6], %					
Very low	14	9	10	.52	.89
Low	59	45	34	<.01	.13
Intermediate	16	17	24	.29	.32
High	7	16	19	.06	.57
Very high	5	13	14	.10	.83
Treatment, %					
Disease/immune MD	22	21	20	.77	.86
Induction chemotherapy	12	24	19	.25	.45
Stem cell transplantation	12	18	23	.10	.43

Table S3A: Clinicopathological features of MDS-E as compared to MDS-NE. *P* value[1]: WHO 2008 MDS-E as compared to MDS-NE. *P* value[2]: WHO 2016 MDS-E - inclusion of AEL - as compared to MDS-NE patients. [3]Equal gender distribution observed among WHO 2008 and 2016 MDS-E and MDS-NE patients. [4]Medical history as described in patients' medical records. [5]Bone marrow fibrosis defined as ≥ grade 2 following the European

Myelofibrosis Network. [6]For MDS-NE: the sum of rounded percentages exceeds 100%. Abbreviations: MD, modifying drugs.

	AEL (n = 26)	AML (n = 59)	(RA)EB (n = 77)	*P* value[1]	*P* value[2]
Clinical characteristics					
Age, years	64 (28 - 76)	65 (23 - 84)	64 (37 - 82)	.80	.43
Female/male, %	38.5/61.5	47.5/52.5	39/61	.44	.96
Medical history[3], %					
Hematological malignancy	4	3	4	.92	.97
Solid malignancy	0	12	11	.067	.08
Auto-immune disorder	4	20	15	.05	.14
Cardiovascular disease	31	20	31	.30	.99
Diabetes mellitus	12	7	11	.46	.90
Peripheral blood findings					
Hemoglobin level, g/dL	10.1 (7.2 - 14.7)	9.8 (6.6 - 12.2)	9.8 (5.6 - 13.8)	.20	.68
Neutrophil count, $\cdot 10^9$	0.4 (0 - 3.2)	0.8 (0 - 16.8)	0.8 (1 - 34.0)	.07	.01
Platelet count, $\cdot 10^9$	45 (5 - 133)	66 (4 - 853)	72 (1 - 362)	.04	.07
Pancytopenia, %	46	34	25	.29	.04
Myeloblasts, %	0 (0 - 17)	3 (0 - 28)	2 (0 - 15)	.02	.13
Erythroblasts, %	0 (0 - 83)	0 (0 - 50)	0 (0 - 69)	.71	.60
Bone marrow morphology, %					
Dyserythropoiesis	96	95	90	.80	.35
Dysgranulopoiesis	29	29	32	.97	.77
Dysmegakaryopoiesis	91	76	86	.15	.53
Multilineage dysplasia	80	75	73	.60	.47
Ring sideroblasts	5 (0 - 98)	14 (0 - 31)	5 (0 - 80)	.72	.38
Myeloblasts, %	9 (3 - 18)	23 (8 - 29)	11 (0 - 19)	<.001	.61
Marrow erythroid cells, %	64 (52 - 82)	24 (3 - 73)	33 (2 - 88)	<.001	<.001
Histopathology, %					
Fibrosis[4]	29	21	42	.53	.27
Hypercellularity	43	60	49	.21	.62
Hypocellularity	24	17	14	.50	.27
Karyotype, %					
Normal karyotype	52	54	54	.88	.88
Complex karyotype	13	20	18	.45	.62
Monosomal karyotype	17	23	11	.60	.44
IPSS-R stratification[5], %					
Very low	0	0	2	NA	.56
Low	15	0	12	.01	.70
Intermediate	20	10	32	.26	.32
High	35	50	32	.26	.78
Very high	30	40	23	.43	.55
Treatment, %					
Disease/immune MD	19	21	22	.85	.79
Chemotherapy	46	44	32	.85	.20
Stem cell transplantation	31	26	30	.67	.98

Table 3B: Clinicopathological features of AEL versus AML and MDS (RA)EB. *P* value[1]: AEL as compared to AML. *P* value[2]: AEL as compared to MDS (RA)EB. [3]Medical history as described in patients' medical records. [4]Bone marrow fibrosis defined as ≥ grade 2 following the European Myelofibrosis Network. [5]For MDS (RA)EB: the sum of rounded percentages exceeds 100%. Abbreviations: MD, modifying drugs.

	Cluster 1: EP (n = 120)	Cluster 2: EP (n = 6)	Cluster 3: MP (n = 6)	Cluster 4 NP (n = 75)	*P* value
Clinical characteristics					
Age, years	65 (22 - 88)	52 (39 - 65)	69 (59 - 76)	65 (32 - 85)	.062
Female/male, %	38/62	17/83	50/50	36/64	.66
Peripheral blood findings					
Neutrophil count, ·10^9	*1.3 (0 - 15)*	*2.1 (0.50 - 4.1)*	*22 (6.2 - 34)*	*1.3 (0 - 7.8)*	*.001*
WBC, ·10^9	*3.2 (0 - 24)*	*4.9 (2.0 - 9.5)*	*52 (33 - 67)*	*3.1 (0.70 - 20)*	*<.001*
Erythroblasts, %	*0 (0 - 22)*	*44 (34 - 83)*	*0 (0 - 5)*	*0 (0 - 7)*	*<.001*
Hemoglobin level, g/dL	9.5 (5.6 - 15)	9.0 (6.6 - 12)	10 (8.2 - 11)	9.8 (5.6 - 15)	.77
Platelet count, ·10^9	93 (1 - 671)	65 (12 - 227)	47 (1 - 276)	80 (5 - 729)	.49
Myeloblasts, %	0 (0 - 17)	1 (0 - 6)	1 (0 - 14)	0 (0 - 13)	.11
Pancytopenia, %	23	17	0	25	.55
Bone marrow morphology					
M/E ratio	*0.77 (0.14 - 1.6)*	*0.45 (0.16 - 1.7)*	*7.8 (1.3 - 51)*	*2.5 (0 - 40)*	*<.001*
Erythroblasts, %	*52 (38 - 88)*	*66 (30 - 86)*	*12 (2 - 40)*	*24 (2 - 37)*	*<.001*
Myeloblasts, %	*2 (0 - 19)*	*3 (0 - 14)*	*10 (0 - 18)*	*4 (0 - 19)*	*.03*
Multilineage dysplasia, %	81	83	33	71	.04
Karyotype, %					
Normal karyotype	54	83	40	57	.48
Complex karyotype	13	17	20	12	.94
Monosomal karyotype	12	17	20	7	.59
Clinical outcome					
OS time, months	52	12	7	33	.077
LFS time, months	not reached	7	7	not reached	.035

Table S4, Identification of patient clusters based on marrow and blood cell counts. K-means clustering analysis was conducted using the variables in Italic font. Two clusters suggestive for erythroid predominance were identified, including an indolent MDS-E subtype (cluster 1) and an aggressive MDS-E subtype (cluster 2). In addition, one patient cluster suggestive for myeloid hyperplasia (cluster 3) and one patient cluster suggestive of normoplasia (cluster 4) were identified. Abbreviations: EH, erythroid predominance; MH, myeloid predominance; NP, normoplasia; M/E, myeloid to erythroid ratio.