Impaired formation of erythroblastic islands is associated with erythroid failure and poor prognosis in a significant proportion of patients with myelodysplastic syndromes

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doi:10.3324/haematol.2015.129015

Supplementary information:

Abbreviations:

ALIP	atypical localization of immature precursor cells
AML	acute myeloid leukemia
BMB, BMBs	bone marrow biopsy, bone marrow biopsies
CMML	chronic myelomonocytic leukemia
del(5q)	5q deletion
Ery-I, Ery-Is	erythroblastic island, erythroblastic islands
FAB	French-American-British
GPC	glycophorin C
Hb	hemoglobin
HbA	hemoglobin A
IPSS	International Prognostic Scoring System
IPSS-R	International Prognostic Scoring System, revised [15]
isolated del(5q)	MDS with isolated del(5q) chromosome aberration
LDH	lactate dehydrogenase
MDS	myelodysplastic syndrome(s)
MDS U	myelodysplastic syndrome, unclassified
MF	marrow fibrosis grade 1 to 3 (MF 1 - 3)
NEC	nucleated erythroid cell(s)
RA	refractory anemia
RAEB	RA with excess of blasts
RAEB/t	RAEB in transformation
RARS	RA with ring sideroblasts
RARS-T	RARS with thrombocytosis
RBC	red blood cell
RCMD	refractory cytopenia with multilineage dysplasia
RCMD-RS	RCMD with ≥15% ring sideroblasts
RCUD	refractory cytopenia with unilineage dysplasia
sq	virtual square of 30.5 µm² projected on a marrow section
sq+	sq with ≥50% NEC
WHO	World Health Organization
WPSS	WHO-based prognostic scoring system

Inclusion criteria of the patients with MDS:

This group comprised a total of 317 patients with MDS monitored and treated in more than 20 hematology centers all over Germany who fulfilled the following inclusion criteria:

(1) All patients had been admitted to the hematology center within the last two decades because of an unclear cytopenia without any history of a life-threatening bone marrow disease, 298 of them without any history of a neoplastic disorder, radiation or chemotherapy. 19 patients who had been cured of a neoplastic disorder by radiation or chemotherapy at least 5 years earlier without any

history of a relapse or a neoplastic involvement of bone marrow, who showed normal peripheral blood findings for more than four years and did not receive any further radiation or chemotherapy after cure of this neoplasia, were included as "therapy-related MDS".

(2) Their bone marrow had been aspirated and biopsied with informed consent by colleagues using BMBs as a standard in the diagnosis of any unclear cytopenia, planning up-front for every patient to have aspirate and biopsy for initial diagnosis of the cause of unclear cytopenia, sending all the BMBs from their patients with cytopenia(s) to our hematopathology department, and

(3) aspirating the bone marrow from all patients with a myeloid neoplasm for cytogenetic evaluation.

(4) The study presented was based on the findings on the day of diagnosis of MDS (BMBs, bone marrow aspirates, peripheral blood findings). Diagnosis of transformation into AML was founded on the detection of ≥ 20 % blasts in blood (differential count) or bone marrow (BMBs and / or bone marrow aspirates).

(5) The diagnostic BMBs and aspirates were adequate for the hematopathologic and cytogenetic analyses. BMBs exceeding 15 x 2 x 2 mm^3 in size with ≥ 10 mm^2 evaluable marrow area in a tissue section and ≥ 3 bone marrow aspirates with a total of ≥ 2,000 nucleated cells, each bone marrow aspirate with ≥ 5 marrow particles, were considered adequate for the morphologic evaluation.

(6) The marrow samples showed an MDS according to the FAB criteria. The diagnosis "RAEB/t" was restricted to cases with MDS-related alterations of maturing hematopoiesis, i.e. AML cases with low blast content without dysplastic changes of residual hematopoiesis were not included. Myelodysplastic/myeloproliferative neoplasms, therapy-related MDS, and patients with 20 – 29 % blasts in blood or bone marrow were not excluded according to the WHO classification since they might be more relevant for erythropoiesis and Ery-Is than the subtypes of MDS. The diagnosis of disease was performed by a hematologist and a hematopathologist on the basis of the morphological findings in bone marrow (BMB and bone marrow aspirate) and peripheral blood. Controversial cases were discussed on the phone or at the microscope. Cases remaining controversial were not excluded from the study, they were classified as "MDS U".

(7) Transfusion of blood components was allowed prior to the diagnosis of MDS (without normalization of peripheral blood cell counts in any patient). In the statistical analyses, only blood cell counts prior to the transfusions were considered. 303 patients were treated by best supportive care (including transfusion of blood components) after diagnosis of MDS as long as the blast content in blood and bone marrow was lower than 20 %, i.e. not by immunosuppressive or immunomodula-

tory regimens, chemotherapy or hypomethylating agents. The diagnostic BMBs from 14 patients with more than 19 % blasts in blood or bone marrow on the day of diagnosis who had been treated by best supportive care (transfusion of blood components, antimicrobial therapy) prior to the diagnosis of MDS (RAEB/t according to the FAB classification) or AML with MDS-related changes (WHO classification) were included in the study.

(8) None of the patients had received growth factors (erythropoietin, G-CSF) within three months prior to the diagnosis of MDS. Application of growth factors (erythropoietin, G-CSF) was allowed after diagnosis of MDS. However, the relevance of the alterations of erythropoiesis observed on the day of diagnosis of MDS for the efficacy of cytokines or growth factors during the further course of disease was not analyzed for statistical reasons since (A) application of and response to these factors were documented less reliably than the onset of transfusion dependency, diagnosis of AML and death of patients, and (B) the criteria for response and failure as well as for termination of the application of the growth factors varied between the hematology centers.

Inclusion criteria for the group with normal healthy marrow:

This group was restricted to persons with

(1) a clinical reason for taking a BMB,

- such as unknown fatigue, unclear pain in bone or joint(s), suspected bone disease (osteoporosis, osteomalacia), transient fever, or unclear transient hematologic changes a few days or weeks earlier, such as lymph node enlargement or alterations of peripheral blood cell counts (transient slight leukocytosis, lymphocytosis, monocytosis or thrombocytosis),
- or at a person's own request (to exclude a hematologic disorder because of relatives with a hematologic neoplasm),

due to ethical reasons because of the risk associated with this invasive technique,

(2) an adequate quality of the BMB and bone marrow aspirates (BMBs: ≥ 15 x 2 x 2 mm^3 in size with ≥ 10 mm^2 evaluable marrow area in a tissue section, bone marrow aspirates: ≥ 5 marrow particles with a total of ≥ 2,000 nucleated cells),

(3) normal healthy marrow in bone marrow aspirates, BMB and immunohistochemical evaluation,

(4) normal peripheral blood cell and differential counts, normal LDH value, normal size of spleen and liver on the day when the BMB was taken,

(5) no evidence of a disorder of bone marrow on the day when the BMB was taken and for a follow-up period of six months after taking the BMB
(6) no evidence of another systemic or neoplastic disease in the past and for a follow-up period of up to six months after taking the BMB, and
(7) no history of radiation or chemotherapy.

Cytogenetic investigations:

Cytogenetic analyses were performed according to standard procedures [1]. Samples providing less than 10 metaphases were excluded. When the bone marrow of a patient was aspirated twice within two months before or after diagnosis of disease with a total number of 10 or more metaphases in both samples, the cytogenetic data from both samples were considered and handled as a single analysis. Description of chromosome aberrations and clone definition followed the recommendations of the International System for Cytogenetic Nomenclature (ISCN) [1]. A complex karyotype was defined as ≥3 aberrations within one clone.

Morphologic evaluation:

From all BMBs, 3 µm thin sections were stained using Maywald-Giemsa, Prussian blue, and Gomori silver impregnation. All cell lines were evaluated for dysplastic features as proposed by the WHO classification (nuclear atypias, dysplastic features of cytoplasms, such as hypogranulation of granulocytes, cytoplasmic PAS positivity of NEC etc., as well as altered ratio of nucleus to cytoplasm of cells). For each cell line, dysplastic features were registered when exceeding 10 % of nucleated cells. Diseases were classified according to the criteria of the FAB group and the World Health Organization (WHO) [2,3] by two experts, a hematologist (bone marrow aspirates) and a hematopathologist (BMBs) or by two hematopathologists. Controversial cases were discussed to come to a final consensus of diagnosis and classification. Three cases which remained controversial were classified as “MDS U”.

Monocytes, promonocytes, and monoblasts were marked by anti-CD68 (PGM1) antibody [4,5]. The antibody PGM1 applied is highly sensitive (100 %) with respect to the identification of macrophages, and we have not as yet observed any patient with MDS whose macrophages were PGM1-negative. This antibody marks a subset of CD68 found on macrophages, monocytic cells (monocytes, promonocytes, monoblasts), plasmocytoid dendritic cells, histiocytic sarcoma and neoplastic mast cells (mastocytosis,

mast cell leukemia), but it does not mark other hematopoietic cells, such as granulocytes, nor other lymphatic (B- or T-) cells as observed with other CD68 antibodies [4].

Abnormal localization of immature precursor cells (ALIP) were defined as clusters or aggregates of ≥ 3 blasts located outside the proliferation zone of granulopoiesis. A case was registered as ALIP-positive when at least three ALIP clusters or aggregates were detected within a BMB section. CD34 and CD117 immunophenotyping were applied to facilitate the detection of ALIPs and blasts and to determine the blast content in controversial cases. Marrow fibrosis (MF) was diagnosed as proposed by the WHO and as described earlier [6,7]. Hypoplasia was diagnosed in cases with a marked reduction in marrow cellularity to a value not observed in age-related cases with healthy marrow.

Any reduction in the number of clusters of ≥5 adjacent NEC to values lower than the 5% percentile observed in healthy marrow was termed “impaired formation of Ery-Is”. Cases with reduced numbers of clusters with ≥5 adjacent NEC, but with loose aggregates of non-adjacent NEC or small goups with <5 NEC without a central macrophage, were called “disruption of Ery-Is”, cases with reduced numbers of Ery-Is, but without loose aggregates or small goups of NEC, were described as “loss of Ery-Is”.

Erythropoiesis was furthermore evaluated for impaired maturation, apoptotic NEC, and Ery-Is. Apoptosis was scored visually whether or not affecting > 1% of erythroid precursor cells. Pyknotic nuclei in terminal normoblasts were excluded from apoptosis counts. Any shift in the proportion of terminal normoblasts to immature erythroblasts and / or proerythroblasts compared to the findings in normal healthy marrow was described as “impaired maturation”. Among cases with an impaired maturation, two groups were distinguished, those with versus without megaloblastoid changes of NEC.

The association of macrophages with Ery-Is was checked indirectly by comparing the counts of $PGM1^+$ macrophages [4,5] sectioned through their nucleus and detected within an Ery-I (serial sections alternatively stained with anti-PGM1 and anti-CD71 or double staining of anti-CD71 with anti-PGM1 or anti-CD169; see Supplementary Figure S1) with the estimated numbers of Ery-Is cut through their center within a tissue section. A relevant dissociation was diagnosed when the counts of Ery-I-associated macrophages fell below 90 % of the estimated numbers of Ery-I centers.

Ery-Is, macrophages, erythroid apoptosis, cellularity, ALIP, and MF were analyzed by evaluating BMBs, and ring sideroblasts by evaluating bone marrow aspirates. All the other morphologic evaluations were done by evaluating both bone marrow aspirates and BMBs. Ery-Is, erythroid apoptosis, impaired maturation of erythropoiesis, percentage of ring sideroblasts, marrow cellularity, evidence of ALIP and MF were evaluated by a single investigator. The results on Ery-Is were controlled by a sec-

ond investigator who was blinded to the results from the first investigator by re-evaluating the BMBs from 214 (exact approach) and 51 (manual counts) randomly selected cases.

Immunohistochemistry:

The following antibodies were applied to mark various hematopoietic cells

antibody against	purchased from:	dilution:	applied to mark the following cell type:
CD34	Immunotech	1 : 50	blasts
CD68: PGM1	Dako	1 : 50	macrophages, monocytes, monoblasts
CD71	Menarini	1 : 80	NEC
CD117	Dako	1 : 50	blasts
CD169	Spring Bioscience	1 : 200	macrophages
GPC	Dako	1 : 100	NEC
HbA	Zytomed	1 : 50	normoblasts

using the BenchMark Ultra staining machine (purchased from Roche).

Statistical analyses:

The chi-square test, analysis of variance, the Mann-Whitney *U* test, uni- and multivariate variance components models, linear and Poisson regression analyses were used to test for differences and correlations. Log-rank test and Cox proportional hazards regression analysis were used to test for the prognostic significance of variables. A result was regarded as “significant” if the probability *P* of the null hypothesis was <0.05.

The contribution of a variable to the degree of anemia was checked by analysis of variance components. The variance component attributable to a variable was estimated by the Methods of Moments. Each variance component was presented as the percentage of the variation of anemia which was estimated as the total variance of the Hb value of peripheral blood minus the normal variation of Hb value (which was determined by the variance of the Hb value of peripheral blood in the control group).

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Supplementary Table S1: Characteristics on the day of diagnosis; *ratio (%) or mean ± standard deviation*

		MDS		normal marrow	
		n	[n = 317]	n	[n = 52]
age	[years]	317	63.0 ± 14.3	52	51.0 ± 19.4
sex	[male]	181	57.1 %	28	53.8 %
hemoglobin	[g / dL blood]	317	9.6 ± 2.3	52	14.1 ± 2.0
anemia	< 14 g/dl (male) or < 12 g/dl (female)	304	95.9 %	0	0.0 %
transfusion	dependency (RBC)[1]	116	36.6 %	0	0.0 %
neutropenia	< 1.8 * 10^9 / L blood	143	45.1 %	0	0.0 %
thrombopenia	< 100 * 10^9 / L blood	122	38.5 %	0	0.0 %
LDH	> 240 U / L blood	76	24.0 %	0	0.0 %
FAB classification	RARS	36	11.4 %		
	RA	109	34.3 %		
	RAEB	82	25.9 %		
	RAEB/t [2]	40	12.6 %		
	CMML	50	15.8 %		
IPSS	low risk	88	27.8 %		
	intermediate-1	118	37.2 %		
	intermediate-2	70	22.1 %		
	high risk	41	12.9 %		
IPSS-R	not applicable [3]	72			
	very low risk	23	9.4 %		
	low risk	79	32.2 %		
	intermediate	53	21.6 %		
	high risk	48	19.6 %		
	very high risk	42	17.1 %		
karyotype:	no aberration (normal karyotype)	179	56.5 %	3	100.0 %
	del(5q) [4]	49	15.5 %		
	-7 [4]	25	7.9 %		
	other chromosome 7 aberrations, t(3q), i(17q) [4]	9	2.8 %		
	+8 [4]	16	5.0 %		
	del(20q) [4]	8	2.5 %		
	-Y (isolated aberration)	4	1.3 %		
	complex, 3 karyotype aberrations	8	2.5 %		
	complex, >3 karyotype aberrations	34	10.7 %		
	further chromosome aberrations	19	6.0 %		
WHO classification	RARS	11	3.5 %		
	RCUD	32	10.1 %		
	isolated del(5q)	19	6.0 %		
	MDS U	3	0.9 %		
	RCMD	66	20.8 %		
	RAEB-1	37	11.7 %		
	RAEB-2	37	11.7 %		
	CMML-1	34	10.7 %		
	CMML-2	27	8.5 %		
	RARS-T	18	5.7 %		
	AML with MDS-related changes [5]	14	4.4 %		
	MDS, therapy-related [6]	19	6.0 %		
special features	ALIP	178	56.2 %	0	0.0 %
	MF (grade 1 to 3)	31	9.8 %	0	0.0 %
	hypoplasia	29	9.1 %	0	0.0 %
WPSS	not applicable [7]	115			
	very low risk	42	20.8 %		
	low risk	51	25.2 %		
	Intermediate	40	19.8 %		
	high risk	53	26.2 %		
	very high risk	16	8.0 %		

[1] ≥ one RBC transfusion per month. [2] 5-29% blasts in the differential count (peripheral blood) or 20-29% blasts in the bone marrow; cases classified as "RAEB/t" according to the FAB classification were classified as "RAEB-2", "AML", or "CMML-2" in the WHO classification. [3] peripheral blood blasts > 19% or white blood cell count > 12 * 10^9 / L or neutrophils > 8 * 10^9 / L or the peripheral blood cell counts were not stable for ≥ 2 months. [4] ± another (not complex) chromosome aberration; cases may also appear in another cytogenetic group. [5] < 30% blasts in blood and bone marrow with MDS of the residual hematopoiesis. [6] patients with history of radiation of chemotherapy, but without history of a neoplastic disease of bone marrow and without evidence of a neoplastic disease other than MDS on the day of diagnosis of MDS. [7] patients with MDS U, CMML, RARS-T, AML, or therapy-related MDS

Supplementary Table S2: Reproducibility of measurements by two investigators: *sq^+ = squares with ≥ 50 % NEC; st.dev. = standard deviation. Manual counts of Ery-Is were poorly reproducible whereas counts of sq^+ showed sufficient agreement. Numerical density and size of Ery-Is were determined by a statistical approach on the basis of the sq^+ counts.*

			1st investigator	2nd investigator	correlation	
method	**procedure (randomly selected cases)**	**n**	mean ± st.dev.	mean ± st.dev.	Pearson	Spearman
manual counts of Ery-Is / mm²	the same sections, the same staining (anti-CD71)	51	60.24 ± 62.18	53.13 ± 43.47	0.440	0.588
counts of sq^+ among 2000 sq	different sections, different stainings (anti-CD71 / anti-HbA)	214	0.153 ± 0.130	0.122 ± 0.118	0.831	0.835
	the same sections, the same staining (anti-CD71)	214	0.153 ± 0.130	0.147 ± 0.125	0.917	0.954

Supplementary Table S3: Alterations of erythropoiesis, differences between MDS and healthy marrow, univariate analyses; *st.dev. = standard deviation*

	MDS (n = 317)		normal marrow (n = 52)		
	mean, %	st.dev.	mean, %	st.dev.	P-value
impaired maturation, not megaloblastoid	60.8 %		0.0 %		P < 0.000005
impaired maturation, megaloblastoid	31.2 %		0.0 %		
dysplastic features (atypias >10% of NEC)	60.3 %		0.0 %		P < 0.000005
apoptosis >1% of NEC	49.5 %		0.0 %		P < 0.000005
Ery-Is size (diameter) [µm]	99.3 ±	21.3	69.6 ±	22.4	P < 0.000005
numerical density within marrow [log(Ery-Is / mm³)]	2.39 ±	0.35	3.11 ±	0.34	P < 0.000005
dissociation of macrophages from >10% of Ery-Is	72.6 %		0.0 %		P < 0.000005

Supplementary Table S4: Influences on Ery-Is (MDS; n=317, univariate analyses); *β_i = Poisson regression coefficient and Δ µm = mean change in diameter by one unit increase of variable; s.e. = standard error*

		numerical density of Ery-Is *(Poisson regression analyses)*		***size*** *(diameter)* ***of Ery-Is*** *(linear regression analyses)*	
		β_i ± s.e.	*P-value*	*Δ µm ± s.e.*	*P-value*
general features:	age [years]	-0.001 ± 0.002	n.s.	-0.057 ± 0.088	n.s.
	sex [male]	0.098 ± 0.073	n.s.	3.285 ± 2.527	n.s.
	Hb level [g / dL blood]	0.031 ± 0.013	P = 0.02	-0.897 ± 0.445	P = 0.04
	transfusion dependency (RBC)	-0.113 ± 0.068	n.s.	3.158 ± 2.374	n.s.
	neutrophils [10^9 / L blood]	-0.008 ± 0.004	P = 0.04	-0.232 ± 0.116	P = 0.05
	thrombocytes < 100 * 10^9 / L blood	-0.008 ± 0.066	n.s.	-2.219 ± 2.032	n.s.
	LDH [U / L blood]	-0.001 ± 0.000	P = 0.008	-0.007 ± 0.006	n.s.
WHO classification:	RCUD	0.565 ± 0.121	P < 0.00001	-1.956 ± 2.776	n.s.
	RARS, RCMD-RS, RARS-T	0.179 ± 0.107	n.s.	17.30 ± 3.77	P < 0.00001
	MDS with isolated del(5q)	-0.256 ± 0.173	n.s.	9.513 ± 5.630	n.s.
	RCMD with <15% ring sideroblasts	-0.116 ± 0.090	n.s.	1.617 ± 3.174	n.s.
	RAEB-1, RAEB-2	-0.243 ± 0.086	n.s.	-0.852 ± 2.861	n.s.
	CMML-1, CMML-2 [1]	-0.187 ± 0.110	n.s.	-5.329 ± 3.600	n.s.
	blast excess ≥ 5 % within marrow	-0.210 ± 0.069	P = 0.002	-3.602 ± 2.476	n.s.
	blast excess ≥ 10 % within marrow	-0.171 ± 0.074	P = 0.02	-2.838 ± 2.678	n.s.
	t-MDS	0.265 ± 0.192	n.s.	-5.885 ± 6.281	n.s.
	AML with MDS-related changes	0.003 ± 0.142	n.s.	2.284 ± 5.559	n.s.
special features:	ALIP	-0.110 ± 0.069	n.s.	-3.649 ± 2.424	n.s.
	hypoplasia	-0.453 ± 0.140	P = 0.001	-0.583 ± 4.556	n.s.
	marrow fibrosis (MF grade 1 to 3)	-0.407 ± 0.085	P < 0.00001	-5.863 ± 3.039	n.s.
	marrow fibrosis (MF grade 2 or 3)	-0.574 ± 0.158	P < 0.00001	-4.197 ± 5.172	n.s.
WPSS:	very low risk	0.227 ± 0.091	P = 0.01	5.539 ± 3.305	n.s.
	low risk	0.103 ± 0.077	n.s.	3.658 ± 2.523	n.s.
	intermediate	-0.071 ± 0.082	n.s.	0.712 ± 3.047	n.s.
	high risk	-0.063 ± 0.076	n.s.	-3.630 ± 2.556	n.s.
	very high risk	-0.220 ± 0.129	n.s.	1.760 ± 4.813	n.s.
IPSS-R:	very low risk	0.118 ± 0.111	n.s.	7.616 ± 4.165	n.s.
	low risk	0.125 ± 0.079	n.s.	1.274 ± 0.742	n.s.
	intermediate	0.175 ± 0.093	n.s.	-3.277 ± 3.104	n.s.
	high risk	-0.377 ± 0.090	P < 0.00001	-1.715 ± 2.862	n.s.
	very high risk	-0.038 ± 0.089	n.s.	-3.537 ± 3.340	n.s.
karyotype:	no aberration (normal karyotype)	0.083 ± 0.073	n.s.	-2.536 ± 2.530	n.s.
	t(3q) [2]	0.344 ± 0.489	not tested [3]	-5.090 ± 16.52	not tested [3]
	del(5q) [2]	-0.061 ± 0.111	n.s.	1.550 ± 3.649	n.s.
	-7 [2]	-0.186 ± 0.120	n.s.	0.560 ± 4.410	n.s.
	del(7q) [2]	0.453 ± 0.697	not tested [3]	-4.183 ± 23.33	not tested [3]
	other chromosome 7 aberrations [2]	0.100 ± 0.392	not tested [3]	-5.183 ± 12.48	not tested [3]
	+8 [2]	0.108 ± 0.138	n.s.	0.630 ± 5.848	n.s.
	i(17q) [2]	-1.538 ± 0.741	not tested [3]	-8.654 ± 21.33	not tested [3]
	del(20q) [2]	0.371 ± 0.228	n.s.	-0.099 ± 7.646	n.s.
	-Y [2]	0.085 ± 0.458	n.s.	34.58 ± 14.97	P = 0.02
	complex >2 karyotype aberrations	-0.012 ± 0.035	n.s.	0.298 ± 1.251	n.s.
	complex >3 karyotype aberrations	0.038 ± 0.101	n.s.	-2.892 ± 4.159	n.s.
	other chromosome aberrations	-0.205 ± 0.121	n.s.	-2.412 ± 5.048	n.s.
erythropoiesis:	impaired maturation	0.144 ± 0.059	P = 0.015	2.251 ± 2.052	n.s.
	dysplastic features (atypias)	-0.026 ± 0.052	n.s.	2.175 ± 1.767	n.s.
	apoptosis >1% of NEC	0.142 ± 0.052	P = 0.009	-1.078 ± 2.002	n.s. [1]
	Ery-Is size (diameter) [* 20 µm]	-0.011 ± 0.002	P < 0.00001		
	numerical density [log(Ery-Is / mm³)]			-11.69 ± 1.037	P < 0.00001
	Ery-Is: dissociation of macrophages	-0.176 ± 0.034	P < 0.00001	2.699 ± 1.664	n.s.

[1] significant in multivariate analysis; [2] ± other chromosome aberration(s); [3] not tested because of too few cases

Supplementary Table S5: Contribution to anemia (MDS, n=317,+ control group, n=52, univariate analyses);
linear regression and analyses of variance components; Δ Hb mean change in Hb value by one unit increase of variable; IPSS-R and WPSS were not considered since anemia / transfusion dependence was included in both variables

		no adjustment			***adjustment** to the difference in the group sizes & stratification according to person's sex*		
		Δ Hb	*± s.e.*	*P-value*	*Δ Hb*	*± s.e.*	*P-value*
general features:	age [years]	-0.023	± 0.010	P = 0.015	-0.041	± 0.009	P = 0.000003
	sex [male]	-0.090	± 0.304	n.s.			
	neutrophils [10^9 / L blood]	-0.001	± 0.016	n.s.	-0.023	± 0.023	n.s.
	thrombocytes < 100 * 10^9 / L blood	-1.083	± 0.307	P = 0.0005	-2.039	± 0.369	P < 0.000001
	LDH [U / L blood]	-0.001	± 0.001	n.s.	-0.003	± 0.001	P = 0.005
WHO classification:	RCUD	-0.510	± 0.532	n.s.	-2.233	± 0.757	P = 0.003
	RARS, RCMD-RS, RARS-T	-0.756	± 0.524	n.s.	-2.471	± 0.743	P = 0.001
	MDS with isolated del(5q)	-1.756	± 0.719	P = 0.018	-3.382	± 1.038	P = 0.001
	RCMD with <15% ring sideroblasts	-0.905	± 0.437	P = 0.039	-2.641	± 0.609	P = 0.00002
	RAEB-1, RAEB-2	-1.386	± 0.365	P = 0.0002	-3.118	± 0.490	P < 0.000001
	CMML-1, CMML-2	-0.445	± 0.435	n.s.	-2.214	± 0.608	P = 0.0003
	blast excess ≥ 5 % within marrow	-1.472	± 0.378	P = 0.000003	-3.257	± 0.378	P < 0.000001
	blast excess ≥ 10 % within marrow	-1.311	± 0.353	P = 0.0002	-3.068	± 0.468	P < 0.000001
	t-MDS	-0.536	± 0.704	n.s.	-2.228	± 1.016	P = 0.03
	AML with MDS-related changes	-0.586	± 0.772	n.s.	-2.272	± 1.118	P = 0.04
special features:	ALIP	-1.338	± 0.298	P = 0.000009	-3.220	± 0.333	P < 0.000001
	hypoplasia	-1.094	± 0.554	P = 0.049	-2.820	± 0.794	P = 0.0004
	marrow fibrosis (MF grade 1 to 3)	-1.961	± 0.432	P = 0.000008	-3.642	± 0.602	P < 0.000001
	marrow fibrosis (MF grade 2 or 3)	-2.241	± 0.657	P = 0.0007	-3.910	± 0.949	P = 0.00005
karyotype:	no aberration (normal karyotype)	1.780	± 0.298	P < 0.000001	3.515	± 0.365	P < 0.000001
	del(3q) [2]	-2.056	± 3.024	not tested [1]	-3.914	± 4.558	not tested [1]
	t(3q) [2]	-3.735	± 2.129	not tested [1]	-5.580	± 3.206	not tested [1]
	del(5q) [2]	-2.070	± 0.443	P = 0.000004	-3.730	± 0.619	P < 0.000001
	-7 [2]	-1.352	± 0.619	P = 0.03	-3.031	± 0.888	P = 0.0007
	del(7q) [2]	-3.584	± 2.130	not tested [1]	-5.429	± 3.208	not tested [1]
	other chromosome 7 aberrations [2]	-4.776	± 2.825	not tested [1]	-6.431	± 4.151	not tested [1]
	+8 [2]	-0.859	± 0.746	n.s.	-2.542	± 1.079	P = 0.02
	i(17q) [2]	-0.868	± 2.008	not tested [1]	-2.529	± 2.944	not tested [1]
	del(20q) [2]	0.140	± 1.013	n.s.	-1.541	± 1.478	n.s.
	-Y [2]	-1.509	± 1.640	n.s.	-3.169	± 2.402	n.s.
	complex >2 karyotype aberrations	-1.035	± 0.483	P = 0.03	-2.747	± 0.681	P = 0.00007
	complex >3 karyotype aberrations	-1.439	± 0.700	P = 0.04	-3.112	± 1.010	P = 0.002
	other chromosome aberrations	0.012	± 1.013	n.s.	-1.667	± 1.478	n.s.
erythropoiesis:	impaired maturation	-1.662	± 0.193	P < 0.000001	-2.580	± 0.178	P < 0.000001
	dysplastic features ≥ 10 % of NEC	-1.820	± 0.285	P < 0.000001	-3.564	± 0.317	P < 0.000001
	apoptosis >1% of NEC	-1.491	± 0.296	P = 0.000001	-3.291	± 0.353	P < 0.000001
	Ery-Is size (diameter) [* 20 µm]	-0.039	± 0.006	P < 0.000001	-0.059	± 0.006	P < 0.000001
	numerical density [log(Ery-Is / mm^3)]	1.170	± 0.138	P < 0.000001	1.568	± 0.118	P < 0.000001
	Ery-Is: dissociation of macrophages	-1.153	± 0.168	P < 0.000001	-2.165	± 0.180	P < 0.000001

[1] not tested because of too few cases; [2] ± other chromosome aberration(s)

Supplementary Table S6: Prognostic relevance (MDS) , univariate analyses;
Cox proportional hazards regression; HR = hazard ratio

		AML-free survival *(n = 300)*			***Risk of transfusion-dependent anemia***[1] *(n = 305)*		
	variables:	*HR*	χ^2	*P-value*	*HR*	χ^2	*P-value*
general features:	age [years]	0.01	2.51	n.s.	0.99	2.21	n.s.
	sex [male]	1.04	0.24	n.s.	0.92	0.92	n.s.
	anemia < 10 g / dL blood	1.69	11.45	P = 0.0007			
	neutrophils [10^9 / L blood]	1.02	11.05	P = 0.0009	1.00	0.10	n.s.
	thrombocytes < 100 * 10^9 / L blood	1.66	16.28	P = 0.0001	1.32	2.70	n.s.
	LDH [U / L blood]	1.00	5.50	P = 0.019	1.00	4.51	n.s.
WHO classification[2]:	RARS	0.46			0.29		
	RCUD	0.50			0.58		
	MDS with isolated del(5q)	0.26			0.78		
	RCMD	0.71			0.76		
	RAEB-1	0.93	149.73	P < 0.00005	1.34	67.22	P < 0.00005
	RAEB-2	1.67			1.98		
	CMML-1	1.23			1.00		
	CMML-2	1.24			1.12		
	MDS/MPN-U, RARS-T	0.22			0.22		
	t-MDS	0.95			0.80		
special features:	ALIP	1.85	25.33	P < 0.00005	1.40	7.31	P = 0.007
	hypoplasia	0.56	4.52	P = 0.03	1.21	0.59	n.s.
	marrow fibrosis (MF grade 1 to 3)	1.88	7.87	P = 0.005	1.35	1.47	n.s. [3]
WPSS [4]:	very low risk	0.39					
	low risk	0.74					
	intermediate risk	0.85	36.15	P < 0.00005			
	high risk	1.21					
	very high risk	2.73					
IPSS-R [4]:	very low risk	0.24			0.26		
	low risk	0.68			0.56		
	intermediate risk	0.97	69.38	P < 0.00005	1.54	45.23	P < 0.00005
	high risk	1.70			1.73		
	very high risk	2.97			2.39		
karyotype:	no aberration (normal karyotype)	0.77	3.06	n.s.	0.62	8.61	P = 0.003
	t(3q) [5]	2.64	2.00	not tested [6]	2.91	2.81	not tested [6]
	del(5q) [5]	0.72	3.72	P = 0.05	1.37	2.08	n.s.
	-7 [5]	3.16	24.00	P < 0.00005	2.36	13.23	P = 0.0003
	del(7q) [5]	17.97	15.04	not tested [6]	4.09	2.78	not tested [6]
	other chromosome 7 aberrations [5]	0.05	0.32	not tested [6]	1.53	0.23	not tested [6]
	+8 [5]	1.38	0.90	n.s.	1.46	1.11	n.s.
	i(17q) [5]	0.75	0.09	not tested [6]	0.05	1.10	not tested [6]
	del(20q) [5]	0.21	3.28	n.s.	0.50	1.32	n.s.
	-Y [5]	0.05	2.02	n.s.	0.05	1.85	n.s.
	complex karyotype aberration	2.26	25.50	P < 0.00005	1.61	5.10	P = 0.02
	complex >3 karyotype aberrations	2.51	19.44	P < 0.00005	1.59	3.91	P = 0.05
	other chromosome aberrations	1.19	1.60	n.s.	1.33	1.19	n.s.
erythropoiesis:	impaired maturation, not megalobl.	1.04	8.53	P = 0.036	0.97	6.59	P = 0.04
	impaired maturation, megaloblastoid	0.64			0.70		
	dysplastic features ≥ 10 % of NEC	2.28	22.27	P < 0.00005	1.86	7.05	P = 0.008
	apoptosis >1% of NEC	0.90	1.46	n.s.	0.84	1.18	n.s.
	Ery-Is size (diameter) [* 20 µm]	0.80	10.57	P = 0.001	1.00	0.01	n.s.
	numerical density [log(Ery-Is / mm³)]	0.64	10.45	P = 0.002	0.47	9.33	P = 0.002
	Ery-Is: dissociation of macrophages	1.76	12.54	P = 0.0004	1.38	11.31	P = 0.004

[1] ≥ one RBC transfusion per month in patients transfusion independent on the day of diagnosis or increase in transfusion dependence by ≥ one RBC transfusion per month in transfusion-dependent patients; [2] the subtypes "MDS U" (AML-free survival , risk of tansfusion-dependent anemia) and "AML with MDS-related changes" (risk of tansfusion-dependent anemia) served as reference category(-ies). [3] significant in multivariate analysis. [4] Cases not classified served as reference category. [5] ± other chromosome aberration(s); [6] not tested because of too few cases.

Supplementary Figure S1: No difference between PGM1 (CD68) and CD169 in marrow macrophage staining:

93-year old male patient with RA; *$CD169^{+}/PGM1^{+}$ macrophages outside and inside of Ery-Is:*