Myelodysplastic Syndromes • Research Paper

Hypochromic red blood cells in low-risk myelodysplastic syndromes: effects of treatment with hemopoietic growth factors

[haematologica]
2004;89:1446-1453

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A B S T R A C T

Background and Objectives. The anemia of low-risk myelodysplastic syndromes (MDS), refractory anemia (RA) and RA with ringed sideroblasts (RARS), may respond to treatment with hematopoietic growth factors (GF)); erythropoietin (Epo) ± granulocyte colony-stimulating factor (G-CSF). The present study was designed to assess whether functional iron deficiency may develop in MDS patients receiving these treatments.

Design and Methods. Erythrocyte scattergrams from 34 patients with RA and RARS (untreated, transfused, or GF-treated with partial or complete erythroid response) were analyzed with Bayer-Advia equipment.

Results. In untreated RARS, the proportion of hypochromic erythrocytes (Hypo-e, median 6.2%, range 1.1-8%) and hypochromic reticulocytes (Hypo-r, median 45%, range 22-48%), as well as mean corpuscular volume (MCV, median 101 fL) were significantly elevated compared to corresponding values in controls. These values increased further after GF-treatment (median 11%, 57%, and 105 fL, respectively), in spite of improved hemoglobin values and adequate body iron stores. The values observed in untreated RA patients largely fell within the normal range, and there was no significant influence of GF treatment. Notably, the hemoglobin content of reticulocytes (MCHr) did not differ between MDS and controls, and was not influenced by GF treatment.

Interpretation and Conclusions. The red cell population in RARS shows morphological abnormalities in terms of varying but overall increased size, and reduced hemoglobin concentration. The proportion of abnormal cells increases after successful pro-erythroid GF treatment, indicating that GF promote erythroblast survival, and maturation into erythrocytes. Hence, the finding of hypochromic red cells should not routinely be interpreted as a marker for Epo-induced functional iron deficiency in MDS.

Key words: myelodysplasia, hypochromic erythrocytes, functional iron deficiency, growth factors.

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Myelodysplastic syndromes (MDS) comprise a heterogeneous group of clonal hematologic stem cell disorders characterized by dysplasia in one or more of the hematopoietic cell lineages, various degrees of peripheral cytopenia, and a risk for progression to acute myeloid leukemia (AML).[1] The main features of the MDS subgroups refractory anemia (RA) and RA with ringed sideroblasts (RARS) are profound anemia, often requiring transfusions, a low bone marrow blast count and a relatively favorable outcome regarding survival and risk of progression to AML.[2,3]

Patients with low-risk MDS show an aberrant pattern of iron distribution in their erythroid bone marrow precursors. Cazzola and co-workers recently showed that the mitochondrial iron of the ringed sideroblasts in RARS is stored in an aberrant form of ferritin, Mt ferritin.[4] Other subtypes of MDS may also display ultrastructural mitochondrial changes including increased iron accumulation.[5] Bowen *et al.* showed that reticulocytes from patients with MDS, and in particular from patients with RARS, were larger and had a lower concentration of hemoglobin than did normal reticulocytes.[6] Erythrocytes were not investigated in this study. Thus, there are several pieces of evidence demonstrating that iron distribution/storage is aberrant in MDS. The underlying causes for this pattern are basically unknown.

About 35-50% of patients with low-risk MDS (RA, RARS and RA with excess of blasts (RAEB) but with <10% myeloblasts) respond to treatment with erythropoietin (Epo) with or without the addition of granulocyte colony-stimulating factor (G-CSF).[7,8] Responses are often durable, with a median duration of response of almost two

years.[7] Relapse of anemia is sometimes linked to disease progression, but more often the bone marrow morphology is unchanged and there is no obvious explanation for the recurrence of anemia.[9]

Patients with renal anemia treated with Epo may develop functional iron deficiency in spite of normal or even increased storage iron.[10,11] These patients need intravenous iron medication to respond to Epo treatment. Functional iron deficiency is defined as an imbalance between iron needs and iron supply of the erythron, and is reflected by an increased proportion of hypochromic erythrocytes, reduced reticulocyte hemoglobin content (MCHr) and microcytic anemia.[10] In 1994 Musto *et al*. described that two Epo-treated patients developed an increased percentage of hypochromic erythrocytes after Epo treatment and that the addition of iron restored the hemoglobin response in a few patients.[12]

Epo±G-CSF treatment is one of the recommended therapies for low-risk MDS with anemia.[13,14] Such expensive drugs should be given in a cost-effective manner. However, in order to do this, we need more knowledge about iron supply and distribution in myelodysplastic erythroid cells. In the present study we show that hypochromic red cells are significantly elevated in untreated RARS, and in certain subsets of RA. The proportion of hypochromic red cells increases dramatically in RARS patients successfully treated with pro-erythroid growth factors, but cannot be considered a marker for Epo-induced functional iron deficiency in these patients.

Design and Methods

Patients

Thirty-four patients with low-risk MDS and 26 healthy controls were included in the study. The MDS patients were divided into six groups according to their current treatment. Among the 18 RA patients six were untreated (RA-0), five were treated with Epo±G-CSF (RA-GF) and seven received erythrocyte transfusions (RA-T). Five of the 16 RARS patients were untreated (RARS-0), eight were GF-treated (RARS-GF) and three were transfused (RARS-T) (Table 1). GF-treated patients were all transfusion-independent and showed a median hemoglobin level of 107 g/L; four had normal hemoglobin values (123-139 g/L) and nine had values ranging from 97-109 g/L. The study was performed according the guidelines of the research ethical committee at Karolinska Institutet and patients and controls gave their informed consent.

Samples and laboratory equipment

The analysis of red cell parameters was performed with an ADVIA 120 hematology system (Bayer Diagnostics, Tarrytown, NY, USA).[15] Samples were collected within a period of two months to ensure a minimal variation in the technical routines. Therefore, no longitudinal samples were collected. Peripheral venous blood samples were collected from all current RA and RARS patients at the Department of Hematology, Huddinge University Hospital, who were able to come to the hospital. The samples, taken into K3 EDTA tubes (Becton Dickinson Vacutainer Systems), were delivered to the Clinical Chemistry Laboratory for analysis within one hour, since unpublished data from the preparatory studies for the method for hypochromic erythrocytes (%Hypo-e) suggested that the fraction of hypochromic cells increased if analyzed more than 3 hours after sampling.

The red cell analysis included B-hemoglobin (B-Hb), erythrocyte indices and reticulocyte indices. The erythrocyte indices measured were mean erythrocyte cellular volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), cellular hemoglobin distribution width (CHDW), red cell distribution width (RDW), hemoglobin distribution width (HDW) and percentage of hypochromic erythrocytes (%Hypo-e) (Table 2). The reticulocyte indices included MCVr, MCHr, MCHCr, CHDWr, RDWr, HDWr and percentage of hypochromic reticulocytes (%Hypo-r) (Table 3). Reticulocyte hemoglobin (MCHr), which has been proposed as a surrogate marker for hypochromic erythrocytes in functional iron deficiency, was of special interest, as was the ratio between MCHr and MCH.[10,16] Body iron stores, liver enzymes, C-reactive protein, leukocyte, neutrophil and platelet counts, lactate dehydrogenase (S-LDH), S-cobalamin and S-folic acid were evaluated using routine clinical methods. In addition, plasma was frozen at -70°C within two hours of sampling, for later analysis of soluble transferrin receptor (S-TfR) and serum methylmalonic acid (S-MMA).

S-TfR were analyzed by nephelometry (Dade-Behring, N latex sTfR, Marburg, Germany) using a BNA Prospec nephelometer (Dade-Behring, Marburg, Germany). S-MMA was determined by isotope dilution gas chromatography mass spectrometry using (2H3)methylmalonic acid as the internal standard, essentially as described by Rasmussen,[17] except that t-butyldimethylsildyl derivatives were used instead of cyclohexyl derivatives.

Control samples

Samples were taken from 26 healthy control subjects at the Clinical Chemistry Laboratory, Karolinska University Hospital, Huddinge to provide reference mate-

Table 1. Patients' characteristics.

	n		Hb (g/L)	WBC (×10⁹/L)	Neutro (×10⁹/L)	Plt (×10⁹/L)	P-iron (μmol/L)	P-ferritin (μg/L)	sTfR (mg/L)	S-MMA (mmol/L)
Normal	26	median	145	6.4	2.9	277	17.5	63	<1.8*	<0.4*
		range	124-170	4.3-10.4	1.5-7.1	180-403	9-32	22-295		
MDS all	34	median	111.5	6.0	3.33	192	27	1074.5	1.52	0.23
		range	73-139	1.3-14.1	0.32-10.8	17-484	9-44	34-12015	0.25-4.66	0.12-0.89
RA all	18	median	116	4.55	2.63	144	24	968	1.31	0.22
		range	76-139	1.3-11	0.32-8.8	17-276	9-41	34-12015	0.25-4.66	0.12-0.89
RA-O	6	median	116	3.65	1.9	179	1.5	175	1.65	0.15
		range	114-136	2.6-6.5	0.32-4.7	92-276	9-24	34-1555	1.13-1.89	0.12-0.44
RA-GF	5	median	121	6.4	4.1	144	21	1183	1.38	0.21
		range	99-139	1.5-8.6	0.36-6.4	99-258	12-29	148-1404	0.74-4.66	0.18-0.45
RA-T	7	median	111	5.2	3.26	36	30	2468	0.86	0.29
		range	76-118	1.3-11	0.69-8.8	17-214	27-41	372-12015	0.25-2.12	0.15-0.89
RARS all	16	median	106	6.65	3.88	302.5	29	1169	2.1	0.23
		range	73-130	4-14.1	2.1-10.8	44-484	13-44	255-4443	0.43-4.52	0.14-0.81
RARS-O	5	median	114	5.7	2.8	298	36	802	1.66	0.17
		range	93-130	4.6-7.1	2.3-3.36	169-408	28-44	255-1471	1.27-2.15	0.15-0.27
RARS-GF	8	median	102.5	8	5.45	335	25	1169	3.36	0.22
		range	97-125	4-14.1	2.7-10.8	44-484	13-38	269-1967	1.19-4.52	0.14-0.81
RARS-T	3	median	92	6.2	3.3	203	36	3775	1.41	0.26
		range	73-112	5-8.8	2.1-7	192-215	33-39	1494-4443	0.43-3.81	0.22-0.34

**Reference values according to laboratory standard methods. n: number of subjects; Hb: hemoglobin content (g/L); WBC: white blood cell count (×10⁹/L). Neutro: neutrophil count (×10⁹/L); PLT: platelet count (×10⁹/L); P-iron: plasma iron (μmol/L). P-ferritin: plasma ferritin (μg/L). sTfR: soluble transferrin receptor (mg/L). S-MMA = Serum methyl malonic acid (mmol/L).*

Table 2. Erythrocyte parameters.

		Normal	RA (all but T)	RA-O	RA-GF	RARS (all but T)	RARS-O	RARS-GF	All transfused
MCV	median	88	102	97.5	102	102	101	104.5	91.5
(fL)	range	81-96	85-113	85-107	96-113	86-117	89-102	86-117	87-110
MCHC	median	349.5	344	349	344	339	344	334.5	348.5
(g/L)	range	334-374	324-375	324-375	325-357	312-355	329-355	312-346	335-373
RDW	median	12.3	15	14.2	16.6	20.8	20.8	20.75	17
(%)	range	11.7-13.3	12.9-20.5	12.9-17.5	13.6-20.5	16.4-25.6	16.4-25.5	17.8-25.6	13.7-20.5
HDW	median	26.65	33.6	32.55	33.6	37.6	34.7	37.8	30.9
(g/L)	range	24.8-35	27.9-35.7	29.6-35.2	27.9-35.7	30.5-41.8	30.5-41.8	35.2-40.5	26.1-51.2
CHDW	median	3.6	5.07	4.88	5.43	8.55	7.49	8.79	4.79
(pg)	range	3.33-4.06	3.56-7.91	3.56-5.44	4.86-7.91	5.28-10.87	6.02-9.65	5.28-10.87	4.02-7.94
MCH	median	30.75	34.1	32.2	34.5	34.2	34.2	34.25	31.1
(pg)	range	29.1-33.7	29.2-38.8	29.2-37.3	30.3-38.8	27.1-39	29.5-35.5	27.1-39.0	28.5-37.4
%Hypo-e	median	0.35	1.6	2.75	1.6	8	6.2	11.2	0.75
(%)	range	0.1-2.1	0.1-12	0.1-11	0.7-12	1.1-18.5	1.1-8	6.5-18.5	0.3-20.5

MCV: mean erythrocyte cellular volume (fL); MCHC: mean cellular hemoglobin concentration (g/L); RDW: red cell distribution width (%); HDW: hemoglobin distribution width (g/L); CHDW: cellular hemoglobin distribution width (pg); MCH: mean cell hemoglobin content (pg); %Hypo-e percentage of hypochromic erythrocytes (%).

Table 3. Reticulocyte parameters.

		Normal	*RA (except transfused patients)*	*RA-O*	*RA-GF*	*RARS (except transfused patients)*	*RARS-O*	*RARS-GF*	*All transfused*
MCVr (fL)	median	104.85	119.25	119	119.5	119.2	116.3	122.1	115.15
	range	99.7-111.4	103.2-128	103.2-127.2	106.3-128	107.4-139.6	107.9-125.7	107.4-139.6	94.3-135.7
MCHCr (g/L)	median	317.5	313.5	317	312	284	286.5	278.5	311
	range	297-327	279-322	279-322	302-320	271-310	284-310	271-300	255-348
RDWr (%)	median	9.45	10.65	10.3	15.4	16.3	14.45	17.8	13.9
	range	8-11	8.5-19.2	8.5-13.6	9-19.2	12.4-22.7	12.4-18.5	13.3-22.7	11.4-16.9
HDWr (g/L)	median	25.45	29.5	29.2	29.8	42.45	40.75	42.95	35.85
	range	20.3-32.5	24-45.4	24-40.2	26.7-45.4	36.6-47.1	36.9-44	36.6-47.1	30.2-41.1
CHDWr (pg)	median	3.26	4.86	4.81	5.16	8.33	7.1	9.43	6.17
	range	2.89-3.93	3.12-7.31	3.12-5.06	4.03-7.31	4.83-10.52	5.67-9.65	4.83-10.52	3.74-7.52
MCHr (pg)	median	33.1	36.2	35.3	36.4	34.05	34.75	33.95	34.85
	range	30.3-35.1	30.3-39.4	30.3-38.4	32.8-39.4	29.2-40.2	30.8-36.1	29.2-40.2	29-41.8
%Hypo-r (%)	median	6.2	13.8	8.4	18.4	47.55	44.5	57.4	17
	range	2.9-20.2	4.4-55.1	4.4-51.1	5.8-24.2	22-63.7	22-48.2	30.5-63.7	3.3-80.7

MCVr: mean reticulocyte cellular volume (fL); MCHCr: mean cell hemoglobin content (reticulocyte) (g/L); RDWr: red cell distribution width (reticulocyte) (%); HDWr: hemoglobin distribution width (reticulocyte) (g/L); CHDWr: cellular hemoglobin distribution width (reticulocyte) (pg); MCHr: mean cell hemoglobin (reticulocyte) (pg); %Hypo-r: percentage of hypochromic reticulocytes (%).

rial for the routine methods. None of the normal subjects were iron depleted (Table 1).

Statistical analysis

The data presented in the tables are expressed as median values±range due to the skewed distribution of many parameters. Results for different groups were compared using the Mann-Whitney U-test and Student's t-test, depending on the distribution of the data.

Results

Baseline investigations

The diagnostic investigations prior to the study revealed normal values for S-cobalamin and B-folic acid in all patients. In spite of this, many of the patients had been treated with vitamin B12 and folic acid for some months before their diagnosis of MDS was established, but without a response. No patient had significant organ failure, three had moderately increased liver enzymes due to iron overload (< 2.5 times upper the normal level), and three had slightly elevated S-creatinine values (<200 mmol/L). No patient showed hemolysis.

Iron status in untreated patients

Serum iron and serum ferritin values were within the normal to high range, indicating adequate or increased body iron stores (Table 1). Median S-TfR levels in untreated RA and RARS patients fell within the reference values (Table 1), with no difference between groups (p=0.81).

Serum methylmalonic acid

Median S-MMA values fell within the reference values (Table 1) and there was no significant difference between the groups. Six patients, however, showed elevated S-MMA levels; four RA patients (one untreated, one successfully treated with GF, and two with pancytopenia and transfusion-dependency), and two GF-treated RARS patients. Five of these had severe disease of many years duration, and four had pancytopenia. The patient with the highest S-MMA level, 0.89 mmol/L, a heavily transfused RA patient, was treated with high doses of cobalamin. No clinical response was observed, and the S-MMA level was unchanged after treatment.

Red cell parameters in untreated patients

Heavily transfused patients showed erythrocyte scattergrams typical for only allogenic erythrocytes and were not considered to have significant endogenous erythrocyte production. As a result they are not included in the statistical analyses of RA and RARS patients, but are displayed separately in Tables 1-3.

Mean erythrocyte cellular volume was higher in MDS patients than in normal controls (p=0.003) (Figure 1, Table 2). Red cell distribution width, hemoglobin distribution width and cellular hemoglobin distribution width

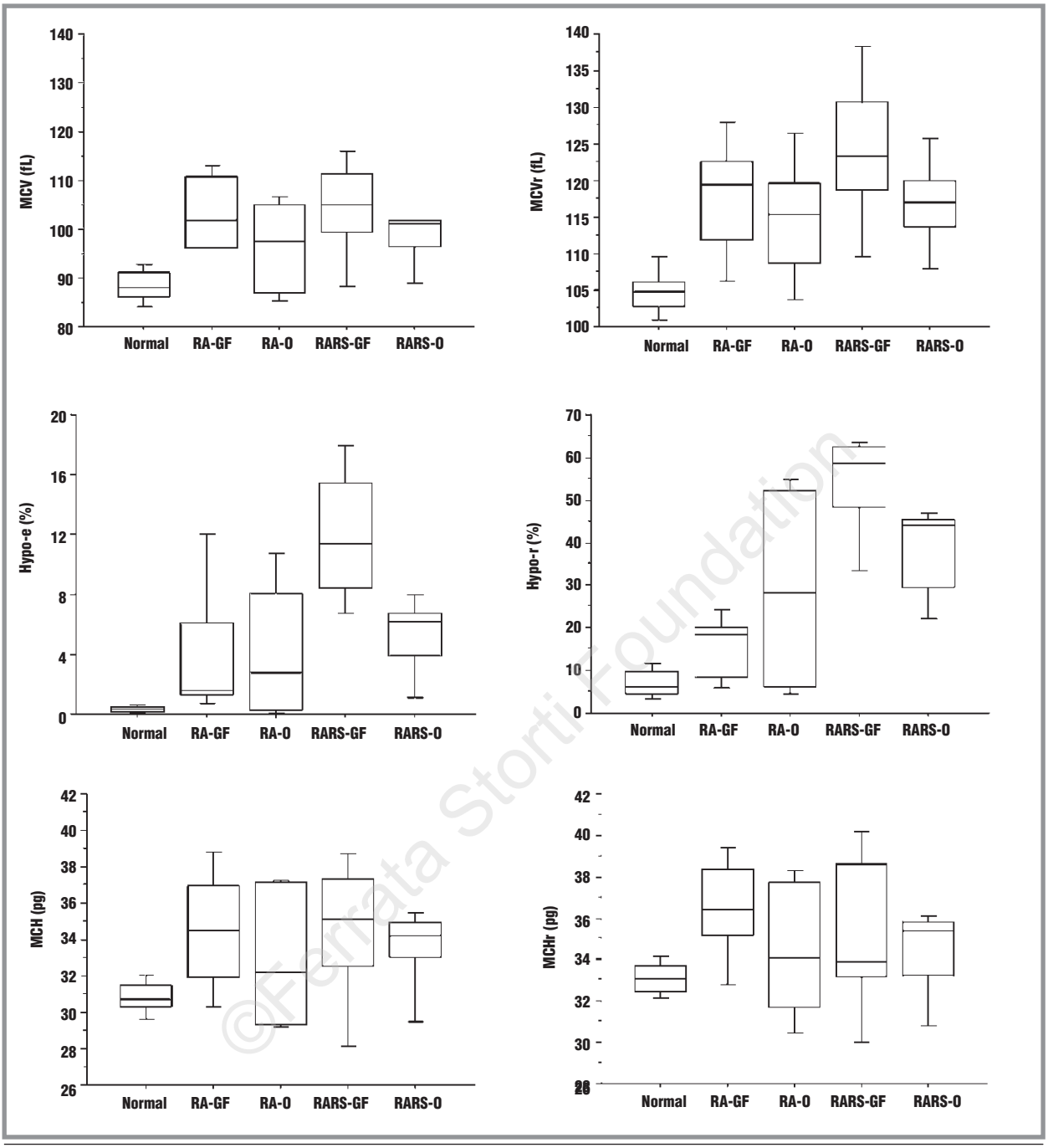

Figure 1. Box plots comparing median values and range for MCV, MCVr, %Hypo-e, %Hypo-r, MCH and MCHr between normal controls and MDS patients with different treatment. Normal: normal controls; RA-GF: RA patients treated with growth factors (eg EPO±G-CSF); RA-0: RA patients with no treatment; RARS-GF: RARS patients treated with growth factors (EPO±G-CSF); RARS-0: RARS patients with no treatment.

were significantly higher in RARS patients (p=0.0005; p=0.002 and p=0.0005, respectively) as well as in RA patients (p=0.0004; p=0.001 and p=0.003, respectively) than in controls. RDW and CHDW were, however, higher in RARS than in RA (p=0.01 and p=0.006, respectively) (Table 2). Mean cell hemoglobin was higher in RARS than in controls (p=0.03), while it did not differ between RA patients and controls (p=0.66) (Table 2). Mean cell hemoglobin concentration of MDS erythrocytes fell within the normal range (Table 2). The percentage of hypochromic erythrocytes was significantly higher in untreated RARS patients than in healthy subjects (p=0.005) while it was not significantly raised in the RA population as a group (p=0.1). %Hypo-e was

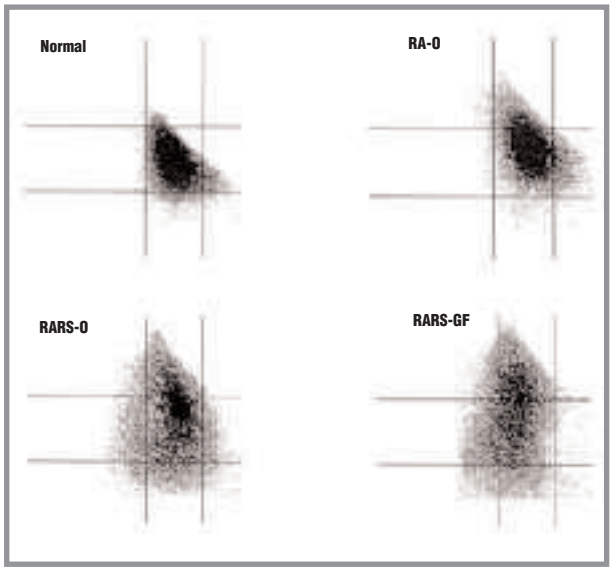

Figure 2. Scattergrams showing the erythrocyte population of one normal control, one RA-0, one RARS-0 and one RARS-GF. Normal: normal control; RA-0: RA patient with no treatment; RARS-0: RARS patient with no treatment; RARS-GF: RARS patient treated with growth factors (EPO±G-CSF).

elevated (≥5%) in all RARS patients but one, and in a third of patients with RA (Table 2) (Figure 1). The erythrocyte scattergram, as exemplified by Figure 1, of untreated RARS patients showed a typical pattern, which confirmed the anisocytosis and anisochromasia. Hypochromic cells varied substantially in size and could be microcytic as well as macrocytic. The erythrocyte scattergrams for RA patients were more similar to those of the controls, in spite of increased parameter variation (Figure 2).

MCVr was significantly higher in MDS patients than in controls ($p<0.0001$) (RA, $p=0.006$ and RARS, $p=0.001$). RDWr, HDWr and CHDWr were higher in RARS patients than in normal subjects. HDWr and CHDWr were also elevated in RA patients (Table 3). In spite of a normal MCHC in MDS erythrocytes, MCHCr was notably lower in MDS patients than in controls ($p<0.001$), with no difference between RARS and RA patients ($p=0.93$) (Table 3). The median MCHr value, although difficult to interpret due to the overall high MCV values,[18] was slightly higher in RARS patients than in controls ($p=0.04$) (Figure 1) (Table 3). The MCHr/MCH ratio was significantly lower in patients with MDS than in controls ($p<0.0001$) and somewhat lower in those with RARS than in those with RA ($p=0.08$), indicating impaired maturation of erythrocytes. The percentage of hypochromic reticulocytes was higher in RARS patients than in controls ($p=0.0005$), while the difference for RA was not significant ($p=0.08$). As for %Hypo-e, the %Hypo-r in RA patients showed substantial variation (Figure 1) (Table 3).

Taking these data together, the increased fraction of hypochromic reticulocytes in MDS reflects a large cell volume in combination with a relatively normal hemoglobin content per cell. Epo±G-CSF treatment induced major changes in the red cell population of RARS patients, while RA patients showed a more varied pattern. Erythrocyte scattergrams showed a higher fraction of abnormal cells for GF-treated RARS patients than for the untreated patients (Figure 2). Marked increases of %Hypo-e ($p=0.008$) and %Hypo-r ($p=0.003$) were noted in GF-treated RARS patients compared to in untreated RARS (Figure 1) (Table 2). Moreover, GF-treated RARS patients showed higher MCVr and lower MCHCr values, but a basically unchanged MCHr compared to untreated cases. Hence, red cells resulting from pro-erythroid GF-treatment are larger but contain the same amount of hemoglobin as cells from untreated patients (Tables 2 and 3, and Figure 1).

TfR levels were slightly higher in GF-treated RARS patients than in untreated RARS patients ($p=0.13$), while there was no difference between GF-treated and untreated RA patients ($p=0.75$) (Table 1).

Discussion

The cellular mechanisms through which hematopoietic growth factors improve the anemia of MDS patients are still basically unknown. We have previously shown that patients responding to treatment have a decrease in the number of apoptotic bone marrow progenitors,[19] but this does not tell us whether treatment has given a selective growth advantage to the normal progenitors or actually allowed dysplastic erythroblasts to escape from intramedullary apoptosis. More recently, we showed that G-CSF suppresses mitochondria-mediated apoptosis of *in vitro* cultured erythroblasts from RARS and RA.[20]

In this study we demonstrated that erythrocytes and reticulocytes from untreated RARS patients showed an increased proportion of cells with a low hemoglobin concentration, although the median MCHC values did not differ significantly from those of the control samples. The reticulocyte results confirm the findings of Bowen *et al.*, while the erythrocyte results are new. The proportion of hypochromic (dysplastic) red cells, as well as MCV values increased significantly. The majority of RA patients did not have an increased percentage of hypochromic cells, but interestingly, the few RA patients who did show this pattern had chromosomal abnormalities, such as monosomy 7 and trisomy 8. This strongly suggests that GF treatment in RARS acts by improving survival of erythroid bone marrow progenitors, thus allowing an increased number of ery-

throblasts to mature into erythrocytes. By contrast, RA patients showed a much more heterogeneous pattern, which was less influenced by GF treatment. The majority of RA patients did not have an increased percentage of hypochromic cells but, interestingly, the few RA patients who did show a high proportion of hypochromic red cells had chromosomal abnormalities, such as monosomy 7 and trisomy 8. Two recent studies have shown that Epo treatment of RA patients with the 5q- aberration and other cytogenetic abnormalities may lead to a decrease in the percentage of cytogenetically abnormal marrow progenitors.[21,22] This might suggest that GF treatment in RA acts by selecting normal or more normal progenitors. The present study constituted a snapshot of untreated, transfused and treated patients, and it was therefore not possible to draw conclusions about effects of GF in individual cases. A longitudinal study analyzing patients before and during GF treatment is warranted.

The erythrocyte appearance varied not just between groups, but also within the erythrocyte population in the individual patient. This feature was particularly characteristic for the RARS subgroup, as illustrated by the scattergram (Figure 2). In combination with the pronounced variation in all red cell parameters this suggests a substantial heterogeneity within the erythropoietic population of RARS patients. Morphologically, erythroblasts in RARS contain a varying amount of Mt ferritin-loaded mitochondria.[4] However, the majority of mitochondria, even in a ringed sideroblast, do not contain morphologically visible iron. We have previously demonstrated that G-CSF may inhibit mitochondria-mediated apoptosis of early erythroblasts.[20] *In vitro*, these inhibitory effects occur prior to the formation of ringed sideroblasts, but clinically it is unclear at what level of maturation erythroblasts could be rescued by GF treatment.

Mitochondrial disturbances have also been reported in RA, but the mechanisms are likely to be different from those in RARS.[5] From a clinical point of view the RA population in our study was more heterogeneous than the RARS population, including patients with various chromosomal aberrations and more patients with severe pancytopenia.

The primary aim of our study was to investigate whether functional iron deficiency may develop in GF-treated MDS patients. Functional iron deficiency, as reflected by hypochromic and microcytic erythrocytes, and low MCHr,[10] is the most common cause of suboptimal Epo response in patients with renal anemia, and can easily be corrected by intravenous administration of iron.[11] The GF-treated RARS patients in our study did indeed have significantly elevated percentages of Hypo-e and Hypo-r, and also elevated S-TfR levels, as a sign of increased erythropoiesis. However, MCV, MCVr, MCH and MCHr were normal or elevated, which does not support functional iron deficiency in these cells.

Moreover, these patients had maintained complete or stable partial erythroid responses, which contradicts a need for additional iron substitution.

We do not exclude that functional iron deficiency may develop in GF-treated MDS patients, and there are anecdotal reports about clinical responses to iron substitution.[12] We do, however, conclude that increased hypochromic reticulocytes and erythrocytes in RARS and in certain RA patients reflects cellular defects leading to macrocytosis, rather than a low iron content in these cells. This interpretation is further supported by the decreased MCHr/MCH ratios observed in MDS. Hence, hypochromic red cells should not routinely be interpreted as a marker for Epo-induced functional iron deficiency in MDS.

MCHr has been suggested to replace%Hypo-e as a diagnostic marker for functional iron deficiency since %Hypo-e values increases with the time from venous puncture to analysis. Our data show, however, that these two markers reflect different biological events in MDS.

The finding of elevated s-MMA values in six of our patients is not easily explained, but does not seem to signify B12 deficiency, either reflected by low S-cobalamin values or response to high doses of B12 treatment. This finding might merit further investigation.

In conclusion, we demonstrate that erythrocyte scattergrams from patients with low-risk MDS, and in particular RARS, show an increased fraction of hypochromic cells which, in combination with adequate iron stores, could be used as a diagnostic marker. We also present evidence that treatment of the anemia of RARS with Epo and G-CSF acts by enhancing erythroid progenitor survival, thus increasing the output of hypochromic erythrocytes. This supports our previous findings suggesting that the erythroid progenitor apoptosis of RARS may be counteracted *in vitro* and *in vivo* by pro-erythroid growth factors.

TL: medical student, took all the blood samples, filed and summarized data, did the statistical calculations and wrote most of the manuscript including the tables and figures; RB: senior physician, is in charge of the Bayer-Advia equipment and interpreted these data; EHL: chairs the MDS program at the department and planned and supervised the study. None of the authors has anything to disclose. These data, or parts of these data, have not been published elsewhere.

Supported by Swedish Cancer Society grant 3689-B01-07XBC and Stockholm Cancer Society grant 01:164.

Manuscript received April 9, 2004. Accepted September 20, 2004.

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