Myelodysplastic Syndromes

Mobilization of hematopoietic progenitors in low-grade myelodysplastic syndromes

Mobilization of hematopoietic progenitors in 15 untreated patients with low-grade myelodysplastic syndrome (IPSS score ≤1) resulted in poor yields in seven patients; moreover, mobilized cells had abnormal cytogenetics and defective *in vitro* growth. Only three out of 15 patients had adequate progenitor cell collections for potential use in autologous transplantation.

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Although still considered as experimental treatment, autologous blood stem cell transplantation in high-grade myelodysplastic syndrome (MDS) has shown encouraging results, with 30% disease-free survival at 4 years[1] Delforge *et al.*[2] demonstrated the re-establishment of polyclonal hematopoiesis in blood stem cells of patients in remission from high-grade MDS.

Autologous transplantation has received little attention in low-grade MDS, but availability of normal hematopoietic cells at diagnosis could offer patients with low-grade MDS a treatment option in advanced phases of the disease. Normal hematopoiesis has been detected in the bone marrow of patients with untreated low-grade MDS.[3,4] However, this has not been investigated in mobilized progenitor cells from patients with low-grade MDS. We studied 15 untreated patients with low-grade MDS (IPSS score ≤1, the low/intermediate-1 risk group)[5] to test whether sufficient CD34$^+$ cells could be mobilized and whether these cells are functionally and cytogenetically normal. MDS was confirmed by examining the blood films, bone marrow aspirates and biopsies, and cytogenetic analyses. Of five females tested by X-linked clonality assays (HUMARA, PGK), three with balanced allele ratios were excluded. The IPSS risk group and WHO category are shown in Table 1. Three patients had hypocellular bone marrow (cellularity < 30%) and two had grade III/ IV myelofibrosis.

Granulocyte colony-stimulating factor (Neupogen, AMGEN, USA), 10 μg/kg/day subcutaneously, was used for stem cell mobilization. Aphereses were performed on a COBE Spectra on days 5 and 6, with the aim of collecting 2×10^6/kg CD34$^+$ cells. Procedures were deferred if the blood CD34$^+$ cell count was < 5/μL.

Unseparated harvested cells were plated in methylcellulose with 100 ng/mL granulocyte-monocyte colony-stimulating factor and 2U/mL erythropoietin. Colonies were scored on day 14. The CD34$^+$ cell analysis was done on a Coulter XL flow cytometer using fluoroscein isothiocyanate-conjugated CD45 and phycoerythrin-conjugated CD34, counting 100,000 CD45$^+$ events. The ratio of colony-forming units, granulocyte-monocyte (CFU-GM) was calculated as CFU-GM/CD34$^+$ cells per volume of harvested cells×100. Results were compared to those obtained in 25 identically mobilized healthy peripheral blood stem cell donors. For cytogenetic analysis, at least 15 mitoses were examined whenever possible.

More than 2×10^6 CD34$^+$ cells/kg were harvested from six of 15 (40%) patients; a single apheresis was sufficient in five out of the six patients (Table 1). In two other patients, 1.46 and 1.60×10^6 CD34$^+$ cells/kg were collected by two aphereses each. Thrombocytopenia precluded a third procedure. In two patients, apheresis was abandoned because of low blood CD34$^+$ cell counts on days 5 and 6 of mobilization (2/μL and 3.5/μL, respectively).

Table 1. Patients' demographics, WHO/IPSS subtype, karyotype, CD34 yield, and *in vitro* culture results.

Patient Sex/Age	*WHO/ IPSS*	*BM karyotype*	*PBPC karyotype*	*CD34 [×10^6/kg]*	*CFU-GM CD34 ratio*	*BFU-E 10^5 MNC*
M/58	RARS/0.5	N	failed	37.8	0.15	0
				23.8	0.16	0
F/44	RC/0.5	N	failed	0.02	n/a	n/a
F/47	5q- Sy/0	del(5q)	del(5q)[87%]	2.62	0.07	0
				2.87	0.38	0
M/19	RC*/0.5	N	N	0.02	n/a	n/a
				0.01	n/a	n/a
M/32	RC*/0.5	N	n/a	n/a	n/a	n/a
M/46	RC/0	N	N	0.74	4.09	2.6
F/32	5q- Sy/0	del(5q)	del(5q)[50%]	3.18	1.52	1.3
M/60	RARS/0.5	N	N	0.51	4.39	6.6
				0.95	4.82	13.3
M/40	RC/0	N	N	0.35	8.8	1.3
				0.6	7.48	0
F/51	RC/0.5	47XX +14	N	2.04	7.45	4
F/52	RC/0.5	47XX +8	47XX+8[77%]	1.25	6.32	10
				0.75	4.15	2.7
F/53	5q- Sy/0	del(5q)	del(5q)[82%]	16.7	2.99	2.4
M/51	RC°/1	Complex	N	1	17.5	10.6
				0.6	10	14.5
F/49	RC°/0	N	n/a	n/a	n/a	n/a
F/18	RC*/0.5	N	failed	0.18	n/a	n/a

**hypocellular bone marrow; °bone marrow fibrosis.*

In vitro culture was performed on 16 harvests collected from ten patients. The mean CFU-GM/CD34 ratio was 4.81±4.15 (95% CI, 1.84-7.79), which was significantly lower than in controls: 9.21±4.11 (95% CI, 7.52-10.90) (p=0.0075, unpaired t-test; Figure 1A). In three patients the CFU-GM/CD34 ratio was < 1.5, whereas the lowest value in 34 donor samples was 1.73. The burst-forming unit, erythroid (BFU-E) count in patients' harvests was markedly lower than in controls: 3.98±4.34 (95% CI, 0.88-7.09) vs 39.22±31.25 (95% CI, 26.02-52.41) (p=0.0013, unpaired T-test; Figure 1B). Two patients had no BFU-E growth, while another four had less than three BFU-E. The lowest value in controls was four BFU-E/10^5 plated cells.

Cytogenetic analysis was performed on bone marrows of all patients at diagnosis and on harvested cells of 12 patients. Of six patients with cytogenetic abnormalities at diagnosis (Table 1), four had the same abnormality in the harvests. In two others, analysis of 5-13 mitoses revealed only normal cells in the harvests.

We empirically chose 2×10^6/kg CD34$^+$ cells as the threshold of adequate collection. Demuynck *et al.*[6] considered 10^6/kg CD34$^+$ cells sufficient for autologous transplantation in MDS, but two of their five patients experienced delayed platelet recovery. Six of our 15 patients had a yield ≥2×10^6/kg CD34$^+$ cells, and two others achieved 1.5×10^6/kg CD34$^+$ cells. Patients with hypocellular bone marrows had low yields of hematopoietic stem cells. Despite good CD34$^+$ cell yields, the harvests of three patients with 5q-syndrome and one with trisomy 8 contained the karyotypic abnormality. Two other patients with abnormal karyotypes at diagnosis had only normal mitoses in the harvested cells, possibly reflecting a low

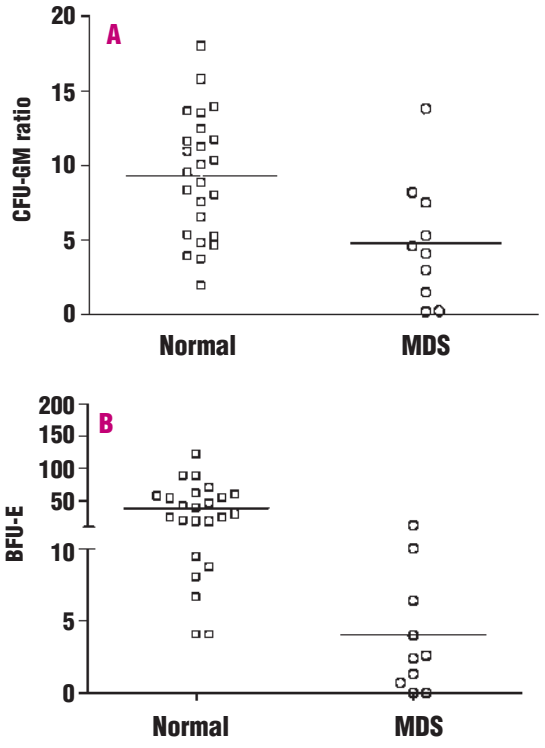

Figure 1. A. The CFU-GM/CD34 ratio is lower in the peripheral blood stem cell harvests from patients with low-grade MDS than in those from controls. When two harvests were performed, the mean value is shown. **B.** BFU-E yield in peripheral blood stem cell harvests is decreased in low grade-MDS. When two harvests were performed, the mean value is shown.

sensitivity of the detection method.

Most MDS patients have poor BFU-E and CFU-GM growth.[7] Six of our patients had absent or very low harvest BFU-E counts, contrasting sharply with the findings in controls. Three patients had low CFU-GM/CD34 ratios. Similar findings, indicative of disordered proliferation of bone marrow $CD34^+$ cells *in vitro*, have been reported in MDS.[8,9]

This study is the first to address the feasibility of collecting mobilized peripheral blood stem cells with normal characteristics from patients with low-grade MDS. If all parameters of *normality* were considered (CD34 count, cytogenetics, *in vitro* colony growth), three out of 15 patients had quantitatively and qualitatively adequate stem cell harvests. Patients with hypocellular MDS failed to mobilize sufficient hematopoietic progenitors, while progenitor cells from those with 5q-syndrome were functionally abnormal. Better understanding of the biology of early stages of MDS is needed to enhance the chance of successful collection of hematopoietic stem cells from patients with low-grade MDS.

Aleksandar Mijovic, Michel Delforge, Maryam Sekhavat, Barbara Czepulkowski, Ghulam J. Mufti*

*King's College Hospital, Department of Hematology, London, United Kingdom; *University Hospital Leuven, Department of Hematology, Leuven, Belgium*

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Correspondence: Aleksandar Mijovic, MBBS, PhD, FRCPath, Dept. of Hematologic Medicine, King's College Hospital, Denmark Hill, London SE5 9RS, United Kingdom.
Phone: international +44.20.73463570. Fax: international +44.20.73464689. E-mail: aleksandar.mijovic@kingsch.nhs.uk

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