A New Method of Mobilization of Hematopoietic Stem Cells in Autologous Stem Cell Transplantation

Kakucs Enikő[1], Benedek I[2], Benedek Erzsébet[1], Köpeczi Judit Beáta[1], Tunyogi Aliz[1], Istrati Monica[1]

[1] Clinical Hematology and Bone Marrow Transplantation Unit, Tîrgu Mureş
[2] University of Medicine and Pharmacy of Tîrgu Mureş

Background: Plerixafor is a selective and reversible antagonist of CXCR4 chemokine receptor indicated in combination with G-CSF (Granulocyte colony stimulating factor) to release haematopoietic stem cells (HSC) into the peripheral blood for collection and subsequent autologous transplantation for lymphoma and myeloma patients.
Objective: We studied the efficacy of the plerixafor in association with G-CSF in poorly mobilizing patients.
Materials and methods: We performed 11 mobilization procedures using plerixafor in combination with G-CSF in 8 patients with Hodgkin's lymphoma, 2 patients with non-Hodgkin's lymphoma and 1 patient with multiple myeloma. The majority of patients have already been heavily pretreated with cytostatic chemotherapy two of them were also treated with radiotherapy. Patients received G-CSF (10 μg/kg/d) for 4 days. On the evening of day 4, they were given plerixafor (0.24 mg/kg) and in the morning we started the apheresis procedures.
Results and discussion: Mobilization with G-CSF and plerixafor was associated with a satisfactory release of CD34+ cells to the peripheral blood as measured 8 hours after plerixafor administration. The final stem cell product contained a median of 3.14×10^6 CD34+ cells/kg (range 1.51–12.1).
Conclusion: Stem cell mobilization with plerixafor and G-CSF provides a solution for majority of patients who were heavily pretreated.

Keywords: plerixafor, poor mobilizer, autologous transplant

Introduction

Transplantation of autologous and allogeneic hematopoietic stem cells is one of the most important curative strategy in patients with hematological malignancies.

Until the late 1980s, bone marrow was the only source of hematopoietic stem cells for transplantation. In the early 1990s, PBSC replaced bone marrow as the preferred source of stem cells because of the relative easiness of the collection, faster engraftment compared with bone marrow transplantation [1].

Only a small number of hematopoietic stem cells circulate in the peripheral blood, mobilization being necessary to mobilize sufficient number of hematopoietic stem cells from the BM to the peripheral circulation, where they can be harvested by apheresis.

Classical strategies for peripheral blood stem cell mobilization include administration of cytokines (Granulocyte-colony-stimulating-factor, G-CSF) alone or in combination with cytostatic chemotherapy (chemomobilization). The action of G-CSF is believed to be based on activation of cells of the neutrophil lineage. These cells secrete proteolytic enzymes that cleave adhesion molecules attaching HSC to bone marrow stroma [2]. Administration of chemotherapy prior to G-CSF provides additional trigger for HSC release from the niche and significantly enhances HSC mobilization [2].

In patients who failed at least one mobilization attempt, a combination of G-CSF and plerixafor may be very effective in mobilizating and collecting hematopoietic stem cells [3].

Plerixafor is a newly targeted drug, which was recently introduced for stem cell mobilization in lymphoma and myeloma patients. Stromal cell-derived factor-1 (SDF-1) is a chemo-kine produced in the stromal cell of bone marrow. This factor induces the migration and homing of hematopoietic stem cells through signaling via the G protein coupled receptor C-X-C4. Plerixafor is a selective and reversible antagonist of CXC4 chemokine receptor and disrupts its interaction with SDF-1, and as a consequence hematopoietic stem cells on release into the blood circulation [4,5,6].

The effectiveness of plerixafor in increasing HSC mobilization suggests the administration of plerixafor in first-line treatment to patients who are predicted to be poor mobilizers. In these cases the mobilization process may be rescued without having to undergo an expensive and time-consuming second mobilization [7].

The generally accepted minimum CD34+ cell yield for transplant is $\geq 2\times10^6$ CD34+ cells/kg, although higher cell doses of $4–5\times10^6$ CD34+ cell/kg or greater are associated with faster neutrophil and platelet recovery, reduced hospitalization, reduced necessity of blood transfusions and antibiotic treatment [8].

In this study we present our experience in the use of plerixafor for hematopoietic stem cell mobilization in 11 patients with hematological malignancies. All of our patients failed previous mobilization attempt with classical mobilization strategies with G-CSF and cytostatic chemotherapy.

Material and methods

We retrospectively analyzed 11 patients, mobilized at the Bone Marrow Transplantation Center in Tîrgu Mureş. The

Table I. Characteristics of the patients

Patient	Age	Gender	Diagnosis	Treatment*	Radiotherapy	Failed previous mobilization regimen
B.C.	38	M	NHL	4 × CHOP-Bleo 4 × R-CHOP+Bleo	Mediastinal	DHAP + G-CSF
D.D	30	F	HL	12 STANFORD courses 3 × DHAP 2 × IPE	–	DHAP + G-CSF
C.L.	30	F	HL	6 × ABVD 6 × BEACOPP	Mediastinal + Supraclavicular	DHAP + G-CSF
N.G.	30	M	HL	2 × ABVD 9 × ABVD 2 × DHAP	–	DHAP + G-CSF
A.V.	42	M	NHL	6 × CHOP 2 × CHOP+Bleo 3 × PROMACE 6 × R-CHOP	–	DHAP + G-CSF
M.R.	52	F	MM	5 × VAD	–	HD-Cy + G-CSF
G.M.	38	F	HL	7 × ABVD 4 × BEACOPP 3 × DHAP 2 × ESHAP 1 × DHAP	Mediastinal + Laterocervical	DHAP + G-CSF
H.N.	21	M	HL	6 × ABVD 1× DHAP	–	DHAP + G-CSF
N.P	26	M	HL	6 × ABVD 2 × DHAP	Mediastinal	DHAP+ G-CSF
K.L.	31	M	HL	6 × ABVD 4 × DHAP	–	DHAP + G-CSF
S.I.	49	M	HL	6 × ABVD 4 × BEACOPP	Subdiaphragmatical	DHAP + G-CSF

M – male, F – female, HL – Hodgkin lymphoma, NHL – non-Hodgkin lymphoma, MM – multiple myeloma
* The abbreviations denote commonly used chemotherapy courses

distribution of these patients by their diagnosis was: Hodgkin's disease in 8 patients, non-Hodgkin lymphoma in 2 patients and 1 patient with multiple myeloma. The median age at mobilization was 35 (range 21–52). Seven patients were males and 4 were females. The majority of our patients were already heavily pretreated and received many chemotherapy courses. Five patients were also treated with radiotherapy before mobilization.

Plerixafor was administered according to the manufacturer's recommendation. For four days the patients received G-CSF at 10 μg/kg body weight 2 times a day. On the evening of day 4, patients received the first subcutaneous injection of plerixafor at a dose of 0.24 mg/kg b.w. The following day the apheresis was performed, repeating the drugs and apheresis for 7–9 days until a sufficient number of CD34+ cells was obtained. The number of nucleated cells and peripheral blood CD34+ cell concentration was assessed by flow cytometry. The decision when to start the plerixafor administration depends on the CD34+ cell count in the peripheral blood during the mobilization process.

Patients underwent apheresis via central venous catheters. The cell collections were performed with Cobe Spectra cell separator, equipped with version 6.1. software. In order to avoid severe hypocalcaemia during the procedure all patients received an intravenous infusion of 10% calcium gluconate.

Vital signs were monitored at the beginning and end of each procedure, and patients were monitored for adverse events during the apheresis procedures. Written informed consent was obtained from all patients for procedural risks.

The CD34+ cells were counted using flow cytometry on the apheresis product prior to processing and cryopreservation.

From the apheresis product, 5 ml samples were incubated with phycoerythrin (PE)-conjugated monoclonal antibody (moAb) anti-CD34, and fluorescein isothiocyanate (FITC)-conjugated moAb anti-CD45. After incubation the red cells were lysed and washed in phosphate-buffered saline. Cells were analyzed by flow cytometer FACScalibur (Becton Dickinson), 100,000 cells for each sample.

The final product containing 10% dimethyl sulfoxide (DMSO) was frozen using a computer-controlled freezer device (Planer) and stored at –196 °C in liquid nitrogen until used.

Results

After analyzing our center's mobilization results we concluded that the combination of plerixafor with cytokines was associated with a satisfactory release of CD34+ stem cells in the peripheral blood. We observed that this type of mobilization regimen was associated with higher peripheral blood leukocytosis. The lower limit of 10 CD34+ cells/μL for apheresis was achieved in 8 of 11 patients. The median number of days of apheresis was 2 days, and it ranged between 1 and 4 days. The processed blood volume was between 10 to 19 L.

While on the day before plerixafor application the median WBC count was 7.9 (range1.2–18.5), it rose to 18.9 (range 2.2–34) after the first plerixafor administration. Median number of peripheral blood CD34+ cell/μL before

Table II. Characteristics of apheresis procedures

Patient	Apheresis	No. of blood WBC count (G/L) 1 day before plerixafor injection	Peripheral CD34+ (cell/µl) counts 1 day before plerixafor injection	No. of blood WBC count (G/L) on the day of apheresis	Peripheral CD34+ (cell/µl) counts after plerixafor administration	Processed volume / apheresis (ml)
B.C.	1	7.9	5.8	11.8	13	15,800
	2			27.2	20	16,000
D.D	1	5.4	4.9	8.9	7.1	12,000
	2			11.4	6.2	12,100
	3			10.9	4.3	12,700
C.L.	1	12	7.9	18.9	25	10,000
N.G.	1	7.8	8.6	11.2	17.4	17,500
	2			10.7	12.3	17,000
A.V.	1	15	8.9	32.0	19.0	16,000
	2			36.8	62.5	16,630
M.R.	1	17.9	7.8	28	11.1	15,000
	2			20	34.0	15,000
G.M.	1	18.5	6.8	34	13.1	14,000
	2			36	12.0	15,000
	3			30	10.0	13,500
H.N.	1	17	4.1	31	6.2	17,500
	2			30	7.5	18,768
	3			28	8.3	17,450
	4			30	3.3	18,500
N.P	1	1.7	6.7	2.2	16.7	19,000
	2			7.8	40.2	18,000
K.L.	1	1.9	3.8	33.5	5.7	16,700
	2			11.1	5.3	17,400
	3			16.6	8.1	17,000
S.I.	1	1.2	7.1	11.7	30.5	18,500

the first injection of plerixafor was 6.58 (range 3.8–8.9), after first application of the drug 13.1(range 5.7–30.5). The median total number of CD34+ cells collected was 3.14×10^6/kg b.w. (range 1.51–12.1).

Two of our patients needed only one plerixafor administration, because the collected number of CD34+ stem cells was sufficient for hematopoietic stem cell transplantation. Three patients from this study received 3, respectively one of them 4 times plerixafor injection. In two patients after the second administration of plerixafor the collected CD34+ cells were unexpectedly high – 6.63×10^6 cells/kg b.w. and 7.75×10^6 cells/kg b.w. respectively.

During the mobilization with plerixafor we did not observe any serious side effects.

All of our patients have already been transplanted and median time of neutrophil (>0.5 G/L) recovery was 12 days (11–14) and for platelets (>20 G/L) it was 14 days (10–15), this being satisfactory.

Discussions

Our results confirm the mobilizing efficiency of plerixafor in lymphoma and myeloma patients who were heavily pretreated and failed a previous mobilization attempt. We observed that in patients who received more than six cycles of chemotherapy the CD34+ cell count was achieved later and showed a lower maximal height of CD34+ cells/L peripheral blood.

A satisfactory circulating CD34+ cell number of ≈10/µL was observed in 8/11 patients after plerixafor administration, which was similar with a Polish group study. They obtained a satisfactory CD34+ cell count in 13/16 patients [9].

Table III. Outcomes of stem cell mobilization and collection

Outcome	Median (range)
CD34+ cells/µL 1 day before plerixafor administration	6.58 (3.8–8.9)
CD34+cells/µL after 1st dose of plerixafor	13.1 (5.7–30.5)
WBC count (G/L) after 1 day before plerixafor injection	7.9 (1.2–18.5)
WBC count (G/L) after 1st application of plerixafor	18.9 (2.2–34)
Number of days of apheresis	2 (1–4)
Total No. of CD34+ cells collected (×10⁶/kg b.w.)	3.14 (1.51–12.1)

Table IV. Results of collection of stem cell

Patient	No. of CD34+ cells × 10⁶/kg b.w. / apheresis	Total No. of CD 34+ cells × 10⁶ / kg b.w.
B.C.	1.3 1.84	3.14
D.D	0.5 0.45 0.315	1.78
C.L.	3.079	3.079
N.G.	1.67 1.143	2.813
A.V.	1.7 6.63	8.33
M.R.	0.825 3.03	3.88
G.M.	1.27 1.23 0.96	3.46
H.N.	0.382 0.517 0.62 0.172	1.69
N.P	1.49 7.55	9.0
K.L.	0.499 0.307 0.708	1.51
S.I.	12.1	12.1

The association between high WBC count and poor CD34+ cell yield was confirmed in another study, being present in our study as well, in two patients who were predicted to be poor mobilizers [10].

The first and largest retrospective study reported an overall success rate of mobilization of 70% after the administration of plerixafor in combination with G-CSF [3]. In our study a minimum number of 2×10^6 CD34+ cells/kg b.w. were collected from 72% of the patients. This rate was similar in the mentioned study.

Conclusions

Combined mobilization with G-CSF + plerixafor enhaced the number of stem cells obtained for autologous hematopoietic stem cell transplantation patients with multiple myeloma and lymphomas.

All of our patients already underwent autologous hematopoietic stem cell transplantation with success we, did not observe altered kinetics of platelet and neutrophil recovery graft failure, the period of aplasia being well tolerated without any serious infections events.

References

1. Stiff PJ – Management strategies for the hard-to-mobilize patient.-Bone Marrow transplantation. 1999, 23: 29–33
2. Bensinger W, DiPersio JF, McCarty JM – Improving stem cell mobilization strategies: future directions. Bone Marrow Transplant 2009; 43: 181–195
3. Calandra G, McCarty J, McGuirk J, Tricot G, Crocker SA, Badel K, Grove B, Dye A, Bridger G – AMD3100 plus G-CSF can successfully mobilize CD34+ cells from non-Hodgkin's lymphoma, Hodgkin's disease and multiple myeloma patients previously failing mobilization with chemotherapy and/or cytokine treatment: compassionate use data. Bone Marrow Transplant 2008; 41: 331–338
4. Gerlach LO, Skerj RT, Bridger GJ, Schwartz TW – Molecular interactions of cyclam and bicyclam non-peptide antagonists with the CXCR4 chemokine receptor. J Biol Chem 2001; 276: 14153–14160
5. Hatse S, Princen K, Bridger G, De Clercq E, Schols D – Chemokine receptor inhibition by AMD3100 is strictly confined to CXCR4. FEBS Lett 2002; 527: 255–262
6. Flomenberg N, Devine SM, DiPersio JF, Liesveld JL, McCarty JM, Rowley SD, Vesole DH, Badel K, Calandra G. – The use of AMD3100 plus G-CSF for autologous hematopoietic progenitor cell mobilization is superior to G-CSF alone. Blood 2005; 106: 1867–1874
7. Stiff P, Micallef I, McCarthy P, Magalhaes-Silverman M, Weisdorf D, Territo M et al. – Treatment with plerixafor in non-Hodgkin's lymphoma and multiple myeloma patients to increase the number of peripheral blood stem cells when given a mobilizing regimen of G-CSF: implications for the heavily pretreated patient. Biol Blood Marrow Transplant 2009; 15: 249–256
8. Pusic I, Jiang SY, Landua S, Uy GL, Rettig MP, Cashen AF et al. – Impact of mobilization and remobilization strategies on achieving sufficient stem cell yields for autologous transplantation. Biol Blood Marrow Transplant 2008; 14: 1045–1056
9. Grzegorz WB, Elzbieta Urbanowska, Magdalena Witkowska, et al – CXCR4 inhibitor plerixafor and G-CSF allow for an effective peripheral blood stem cell collection in patients who failed previous mobilization attempt. Translational Biomedicine 2010, 1:1–8
10. Fowler CJ, Dunn A, Hayes-Lattin B, Hansen K, et al. – Rescue from failed growth factor and/or chemotherapy hsc mobilization with G-CSF and plerixafor (amd3100): institutional experience. Bone Marrow Transplant 43(12) 2008: 909–917