

Original article

# Prospective identification of potential factors influencing stem cell mobilization and the necessity for plerixafor use in newly diagnosed multiple myeloma patients undergoing autologous stem cell transplantation



Gopal Krushna Ray , Rabindra Kumar Jena \*, Tribikram Panda , Sudha Sethy

SCB Medical College, Cuttack, Odisha, India

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ABSTRACT

**Introduction:** To study the efficacy and safety of single large volume leukapheresis by using generic G-CSF or G-CSF plus Plerixafor in achieving adequate stem cell yield and various factors influencing thereof in newly diagnosed multiple myeloma patients undergoing autologous stem cell transplant.

**Method:** This prospective study was undertaken among 55 newly diagnosed multiple myeloma patients undergoing autologous stem cell transplant and aged between 18 and 75 years. Mobilization and harvesting of stem cells were performed by using GCSF or GCSF plus Plerixafor and large volume leukapheresis, respectively. A stem cell yield of  $\geq 2 \times 10^6 \text{ kg}^{-1}$  and the number of apheresis procedures were primary efficacy endpoints, while the ideal stem cells yield  $> 5 \times 10^6 \text{ kg}^{-1}$ , the engraftment day and D100 response/graft sustainability were secondary endpoints.

**Result:** The primary endpoint was achieved in all cases in both the groups by using a single LVL leukapheresis procedure. Fulfillment of all the secondary endpoints was satisfactory and comparable in both the groups. Age, pre-apheresis CD34+ count and number of interruptions during the LVL were significant factors influencing the stem cell yield ( $p < 0.05$ ). Adverse drug reactions during the apheresis and post-ASCT period were manageable.

**Conclusion:** The LVL is safe and cost-effective in attaining a minimum of CD34+ cells in a single procedure with manageable adverse reactions. Judicious intervention during the procedure may be helpful in ensuring the adequate yield.

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\* Corresponding author at: Professor and Head, Dept Of Clinical Hematology, SCB Medical College, Cuttack 753007, Odisha, India.

E-mail address: [rjkjena@msn.com](mailto:rjkjena@msn.com) (R.K. Jena).

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## Introduction

High-dose chemotherapy (HDT), followed by autologous stem cell transplantation (ASCT), has remained the standard of care for multiple myeloma (MM) for the last three decades, as it gives a better survival advantage, compared to chemotherapy (CT) alone.<sup>1,2</sup> Adequate CD34<sup>+</sup> stem cell mobilization is an important pre-requisite step for the successful outcome of ASCT. However, approximately 20% of the patients with MM are poor mobilizers, either with cytokines alone or cytokines and chemotherapy.<sup>3</sup> Repeated CT, prior radiotherapy, old age, advanced disease with extensive marrow involvement and altered bone marrow milieu are known limiting factors for successful mobilization due to compromised stem cell (SC) reserve. Multiple mobilization attempts in these patients is not only a cumbersome procedure, but also increases the transplantation cost, which may be a limiting factor for many patients in a developing country.<sup>4</sup> Approval of Plerixafor for stem cell mobilization is a landmark development that has enhanced the success rate of adequate stem cell yield.<sup>5,6</sup> It is the selective chemokine receptor CXCR4 antagonist facilitating the mobilization of stem cells from bone marrow to peripheral blood.<sup>7,8</sup> Availability of generic Plerixafor with low cost has improved the affordability. This Institution has successfully mobilized stem cells with adequate yield in a single setting apheresis and transplanted them into elderly patients with MM (>65 years) with the help of generic Plerixafor for first time in India.<sup>9</sup> Conventional hematopoietic cells collected by apheresis (HPC(A)) typically involves the processing of two to three blood volumes of the patient/donor, whereas large volume leukapheresis (LVL) processes three to six total blood volumes (up to 20–36 L of blood).<sup>10,11</sup> The LVL procedure takes more time, entails a greater loss of platelets and increases the possibility of side effects, but is cost-effective in comparison with the conventional HPC(A) procedure. Thus, the LVL procedure may be affordable to most of the patients in resource-constrained settings.<sup>12,13</sup> One Indian study has compared the extraction efficiency of the LVL procedure performed in a non-uniform cohort (MM, Hodgkin's disease, non-Hodgkin's lymphoma, germ cell tumor and amyloidosis) of patients undergoing an ASCT by using the granulocyte colony stimulating factor (G-CSF) alone.<sup>14</sup> This was a retrospective analysis of factors influencing the stem cell yield, without any reporting on safety and post-ASCT parameters, such as engraftment or D+100 graft sustainability.

The present prospective study was undertaken with the following objectives:

1. To determine the efficacy and safety of the LVL procedure in achieving adequate stem cell yield in newly diagnosed MM (NDMM) patients by using generic G-CSF, with or without Plerixafor.
2. To investigate the various factors influencing the stem cell yield.

## Material and methods

This prospective non-randomized study was undertaken in the Department of Clinical Hematology, SCB Medical College and Hospital, India, over the last 3 years (2014–17). Fifty-five consecutive NDMM (according to the International Myeloma Working Group Revised Diagnostic Criteria, 2014), qualified and willing to undergo ASCT at our center, were included in the study.<sup>15</sup> The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and approved by the institutional ethics committee. All patients provided written informed consent.

### Study population

#### Inclusion criteria

Patients with NDMM between 18 to 75 years of age achieving satisfactory therapeutic response ( $\geq$ very good partial response (VGPR), according to the revised uniform response criteria by the International myeloma working group, 2016), following three to six cycles of VTD (Velcade/bortezomib, thalidomide, dexamethasone), and willing to receive ASCT were included in the present study.<sup>16</sup> Other essential criteria included were: performance status more than 70% (Karnofsky scale), satisfactory organ function (cardiac, hepatic, renal and pulmonary), negative viral markers (HBV, HCV, HIV, CMV, EBV and herpes). Women of child-bearing age group agreeing to submit consent for contraception were also included in the study. Comorbidities, such as hypertension and diabetes, were not considered as contraindications for inclusion.

#### Exclusion criteria

Patients were excluded if they had a creatinine clearance  $<30$  mL/min, aspartate transaminase (AST)  $\geq 2$  times the upper normal limit, cardiac ejection fraction  $\leq 55\%$  or abnormal pulmonary function test FEV1/FEV  $<70\%$ . Patients who received 2 or more alkylating agents, a total dose of  $\geq 200$  mg/m<sup>2</sup> of prior melphalan as a part of CT, granulocyte colony-stimulating factor (G-CSF) within 14 days before the first dose of G-CSF for mobilization, failed previous hematopoietic stem cell collections or collection attempts or had prior radiation therapy, any comorbidities rendering them at high risk for treatment complications or acute infections, were excluded from the study. Female patients testing positive to a pregnancy test or lactating females were also excluded.

#### Treatment plan and transplantation

All of our patients were evaluated with detailed history, general and systemic examinations, hematologic and biochemical parameters, bone marrow study, serum protein electrophoresis and immunofixation, estimation of serum-free light chain (FLC) ratio, urine analysis and 24 h urinary protein estimation and skeletal survey (X-ray and MRI), as per the standard protocol. All cases received uniform induction CT (3–6 cycles of bortezomib, thalidomide and dexamethasone and, depend-

**Table 1 – Bortezomib, thalidomide and dexamethasone protocol (VTD).**

Chemotherapy drug	Dose and schedule
Bortezomib	1.3 mg/m <sup>2</sup> , IV infusion, weekly for 4 weeks
Thalidomide	200 mg/day, oral until end of induction CT
Dexamethasone	40 mg once a week, oral until end of induction CT

ing on the response, each cycle was of a 4-week duration), as presented in **Table 1**, along with other supportive measures.

After achievement of a satisfactory response ( $\geq$ VGPR), a detailed pretransplant evaluation was made in all cases, as per inclusion/exclusion criteria. Detailed counseling and other procedures were followed, as per the standard protocol.

#### Stem cell mobilization

After satisfactory recovery from the last induction CT (white blood count  $>2.5 \times 10^9 \text{ L}^{-1}$ , an absolute neutrophil count  $>1.5 \times 10^9 \text{ L}^{-1}$  and a platelet count  $>100 \times 10^9 \text{ L}^{-1}$ ), the G-CSF was administered subcutaneously once daily each morning (prior to harvest on days of apheresis until completion of harvest) at 8:00 a.m. at a dose of 10  $\mu\text{g}/\text{kg}/\text{day}$  from D1 to D5. The total leukocyte count (TLC) and CD34 count were evaluated on D4 at 10:00 a.m. by the fully automated cell counter Sysmex XT and BD FACSCanto™ II, respectively. On D4 the CD34 count was estimated from the peripheral blood 2 h after the G-CSF dose. If the CD34 count was  $\geq 10 \mu\text{L}^{-1}$  on D4 and/or D5, stem cell apheresis was performed on D5 by the COBE spectra apheresis equipment. If the CD34 count was less than  $10 \mu\text{L}^{-1}$  on D4, Plerixafor (0.24 mg/kg if creatinine clearance (Crcl) was  $\geq 50 \text{ mL/min}$  or 0.16 mg/kg if the Crcl was 30–49 mL/min) was administered subcutaneously (11:00 p.m. on D4), followed by G-CSF at the usual dose at 8:00 a.m. on D5. The CD34 count was re-estimated on D5 at 10:00 a.m. by flow cytometer. If the CD34 count was  $\geq 10 \mu\text{L}^{-1}$ , apheresis was immediately performed on same day. If the CD34 count on D5 was less than  $10 \mu\text{L}^{-1}$ , another dose of Plerixafor was given at 11:00 p.m. on D5, followed by G-CSF and stem cell enumeration on D6, as mentioned earlier. If the CD34 count on D6 was  $\geq 10 \mu\text{L}^{-1}$ , apheresis was performed. If the CD34 count on D6 was less than  $10 \mu\text{L}^{-1}$ , apheresis was postponed. The stem cell apheresis procedure was repeated on the second consecutive day if the stem cell yield was  $<2 \times 10^6 \text{ CD34}^+$  cells/kg.

#### Apheresis procedure

All the LVL processes were performed with the COBE spectra cell separator, as per the manufacturer instructions, and the software version 7.0, which has a higher CD34 yield and better collection efficiency. The settings for LVL were adjusted to the mean yield of  $5 \times 10^6 \text{ CD34}^+$  cells/kg (predicted value) with a low extracorporeal volume of 285 mL, so that no blood component would be required during the procedure. The average anticoagulant citrate dextrose (ACD) solution to blood flow rate was 1:14. The vitals of the patient were monitored hourly. All of the patients were given prophylactic oral calcium tablets every 30 min during the procedure. Stem cell collection by apheresis was conducted, as per the manufacturer's guidelines, and started after the mononuclear cell collection tubing matches with the provided colorigram. If there was any interruption

of the procedure due to non-cooperation, bathroom urgency, rigor, blockage of access/return line, etc., or any other reason, the procedure was restarted by following standard operating procedures. One dedicated apheresis expert was always in vigilance to modify the plasma flow rate, as per the need to match the color of the collection line with the colorigram. After completion of the apheresis, the complete blood count (CBC), CD34 count estimation and microbiological culture of the product were made. The stem cell yield (actual value) was calculated by using the following formula:

$$\text{Yield} = \frac{\text{Post - CD34 count} \times \text{product volume ml}}{\text{body weight in kg}}$$

The goal of the stem cell collection was  $5 \times 10^6 \text{ CD34}^+$  cells/kg, with a minimum dose of  $2 \times 10^6 \text{ CD34}^+$  cells/kg. The collected stem cells were stored at 2–8 °C in a blood bank refrigerator and infused into the patient on the next day.

$$\begin{aligned} \text{Collection efficiency} &= \frac{\text{Leukapheresis Product CD34 count}}{\text{TLC} \times \text{Product volume ml}} \times \\ &\quad \frac{\text{Pre - procedural CD34 count}}{\text{TLC} \times (\text{Total volume processed} - \text{ACD volume})}. \end{aligned}$$

#### Conditioning regimen

All were conditioned with high dose melphalan (200 mg/m<sup>2</sup>, 140 mg/m<sup>2</sup>, if the glomerular filtration rate (GFR) were less than 50 mL/min/1.73 m<sup>2</sup>) at 9:00 p.m. on the day of the satisfactory stem cell yield by following standard procedures and supportive measures (hydration, antiemetic, ice suckling, etc.). Stem cells were infused intravenously (IV) on the next day at 12 noon. The G-CSF (5 mg/kg/day) was administered from the fifth post-transplant day until engraftment. Platelet and red cell support were provided whenever necessary. Levofloxacin, acyclovir and fluconazole were used as infection prophylaxis, along with other supportive treatment.

Neutrophil engraftment was defined as the neutrophil count  $\geq 0.5 \times 10^9 \text{ L}^{-1}$  for 3 days or  $\geq 1.0 \times 10^9 \text{ L}^{-1}$  for 1 day. Platelet engraftment was defined as the platelet count  $\geq 20 \times 10^9 \text{ L}^{-1}$ , without a transfusion for the preceding 7 days. Graft sustainability on D100 was defined as the maintenance of blood counts according to at least 2 of the following 3 criteria: a platelet count more than  $50 \times 10^9 \text{ L}^{-1}$ , without transfusion for at least 2 weeks before the follow-up visit, a hemoglobin level  $\geq 10 \text{ g/dL}$ , with no erythropoietin support or transfusions for at least 1 month before the follow-up visit, absolute neutrophil count  $>1 \times 10^9 \text{ L}^{-1}$ , with no G-CSF for at least 1 week before the follow-up visit. Patients were discharged 3 days after engraftment and were advised to undergo regular follow-up for evaluation. Subsequently, on D + 100 all patients were evaluated for the status of MM (M protein, free light chains, serum protein, serum calcium, Beta 2 microglobulin and serum lactate dehydrogenase (LDH)) and graft sustainability, as mentioned earlier. From D + 101, all of

the patients received maintenance CT with either lenalidomide at 10 mg/day or bortezomib at 1.3 mg/m<sup>2</sup> subcutaneously every 2 weeks until progression or unacceptable toxicities.

### Efficacy and safety assessments

The primary efficacy endpoints were the number of patients achieving minimum adequate stem cell yield ( $2 \times 10^6$  CD34<sup>+</sup> cells per kg) and number of apheresis processes achieving this. The secondary efficacy endpoints were the number of patients achieving ideal stem cell yield ( $\geq 5 \times 10^6$  CD34<sup>+</sup> cells/kg), the engraftment day, safety of the procedure, well-being of the patients during apheresis and graft sustainability on D100.

The safety of the LVL was monitored by observing vital signs, clinical/laboratory parameters and adverse events/reactions (ADRs) during the procedure. Adverse events occurring up to 100 days after the ASCT were documented.

### Statistics

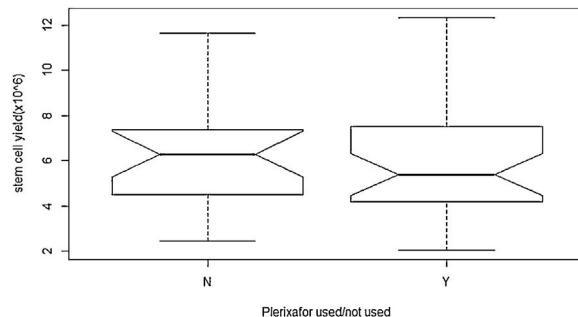
Categorical data were summarized in frequency tables by the number and percentage of patients falling into each category. Continuous data were summarized using descriptive statistics, including the number of observations, mean, standard deviation, median and minimum and maximum values. A linear regression analysis was performed to evaluate the impact of patient/donor age, gender, BMI (body mass index), BSA (body surface area in m<sup>2</sup>), hemoglobin (Hb), leukocyte count, platelet count, Karnofsky PS, pre-apheresis CD34 count, type of pre-transplant myeloma response (stringent complete response (sCR) vs. VGPR) and interruption of the LVL procedure (for reasons, such as non-cooperation, bathroom urgency, rigor, blockage of access or return line, etc.) as independent variables, with stem cell yield as the dependant variable. A *p* value of  $< 0.05$  was considered statistically significant. All statistical analyses and calculations were made using the trial version of SPSS version 19.

### Observation

A total of 55 cases of NDMM (35 male and 20 female) were included in the present study. The baseline characteristics and post-induction myeloma status are presented in Table 2. Forty-seven cases (85%) achieved sCR/complete response (CR), while rest achieved VGPR after the induction phase of the treatment. Details of stem cell mobilization, conditioning regimen, engraftment and post-ASCT response (+100 days) are shown in Table 3. Successful stem cell mobilization (primary efficacy endpoint) was achieved in all cases by a single LVL procedure; 21 cases with G-CSF alone and 34 cases with G-CSF plus Plerixafor. Interruption of the procedure due to various reasons (non-cooperation, bathroom urgency, rigor, blockage of access/return line, etc.) was seen in 6 cases and 17 cases in the G-CSF and G-CSF plus Plerixafor groups, respectively. The ideal stem cell yield of  $\geq 5 \times 10^6$  kg<sup>-1</sup> CD34<sup>+</sup> was achieved in 66.7% of the cases in the G-CSF group, vis-a-vis in 61.7%, in the G-CSF plus Plerixafor group, albeit higher median pre-apheresis CD34<sup>+</sup> count in the latter. Although there was a

**Table 2 – Demographic and baseline characteristics of the patients (n = 55).**

Age in years	52 (37–74)
Sex (M: F)	35: 20
Hemoglobin (gm/dL)	9.2 (7.8–14)
Total Leukocyte Count (/mm <sup>3</sup> )	10,200 (3646–22,860)
Total Platelet Count ( $\times 10^9$ L <sup>-1</sup> )	130 (98–337)
Myeloma subtype n (%)	37 (68%)/10 (18%)/3 (5%) (IgG-kappa/IgG-lambda/IgA-kappa)
Light chain myeloma (kappa/lambda)	2 (4%)/3 (5%)
S.FLC	4.37 (0.009–93.04)
S.Calcium (mg/dL)	10.59 (8–14.2)
S.Creatinine (mg/dL)	1.3 (0.9–5.2)
Lytic bone lesions (%)	23 (41.81%)
ISS Stage of MM (I/II/III)	8 (14%)/23 (42%)/24 (44%)
M component (g/dL)	4.90 (2.6–10.8)
S Total Protein (g/dL)	7.84 (4.72–10.8)
S Albumin (g/dL)	3.07 (2.3–3.6)
S LDH (u/L)	1018.03 (284–1418)
S Uric Acid (mg/dL)	6.17 (3.8–9.7)
Induction CT	3–6 × BTD for all cases
Post-induction status (sCR/CR/VGPR)	9 (16%)/38 (69%)/8 (15%)



**Figure 1 – Effect of plerixafor on stem cell yield.**

significant increment in the CD34 count after the utilization of Plerixafor, there was no significant difference in the SC yield between these two groups (*p* = 0.3982). (Figure 1) Standard high-dose melphalan was used as the conditioning regimen in both groups. Neutrophil/platelet engraftment, D100 myeloma status and graft sustainability were the same in both groups, i.e., 12 days vs. 16/15 days, 90%/100% sCR and 95%/97%, respectively. (Table 3)

Table 4 depicts the linear regression model, in which the adjusted R<sup>2</sup> = 0.560 showed that the 56% variance in the dependant variable, i.e., the stem cell yield, can be explained conjunctively by all the independent variables, such as age, gender, BMI, BSA, hemoglobin, leukocyte count, platelet count, Karnofsky score, pre-apheresis CD34 count and no interruption during apheresis. The number of interruptions during the procedure, pre-apheresis CD34 count and age of the patients were found to be significant factors (*p* < 0.05), in increasing order, influencing the stem cell yield in the regression analysis (Table 4). The ADRs were reported in 39 cases (71%) during

**Table 3 – Stem cell mobilization, conditioning regimen, engraftment and post-ASCT response.**

Stem cell enumeration CD34 ( $\mu\text{L}^{-1}$ )	GCSF group (n = 21)		GCSF + Plerixafor group (n = 34)
	On D4/D5	On D4/D5	Post-Plerixafor
No. of LVL	28.15 (19.85–37.22) One (all cases)	8.385 (6.70–10.14) One (all cases)	30.40 (6.41–35.70)
Volume processed	11,103 mL (7650–15138 mL)		
Collection Efficiency (CE)	37.8% (8.7–182.3)		
Procedure duration	330 min (285–350 min)		
Median no. of interruptions during LVL procedure	3 (6 cases)	3 (17 cases)	
Yield (n)	$\geq 2 \times 10^6 \text{ kg}^{-1}$ $\geq 5 \times 10^6 \text{ kg}^{-1}$	7 (12.72) 14 (25.45)	13 (23.63) 21 (38.18)
Conditioning regimen	Melphalan 200 mg/m <sup>2</sup>	20 (95%)	33 (97%)
Engraftment days	Melphalan 140 mg/m <sup>2</sup> Neutrophil Platelet	1 (5%) +12 (11–14) +16 (15–18)	1 (3%) +12 (11–14) +15 (15–18)
D100 Evaluation			
Myeloma status	19 (90%)	All sCR	
Graft sustainability (n)	20 (95%)	33 (97%)	

**Table 4 – Factors affecting LVL.**

Independent variables	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
	B	Std. Error				Lower Bound	Upper Bound
Age	−.100	.031	−.439	−3.205	.003	−.164	−.037
Gender	−.978	.605	−.207	−1.617	.113	−2.197	.241
BMI	−.008	.078	−.012	−.105	.917	−.165	.148
BSAm2	−3.449	2.192	−.230	−1.573	.123	−7.866	.969
Hemoglobin	.033	.162	.021	.205	.839	−.293	.360
Leukocyte count	.091	.054	.179	1.686	.099	−.018	.201
Platelet_count	.481	.479	.098	1.003	.321	−.485	1.446
Karnofsky PS	.013	.064	.021	.211	.834	−.115	.142
Preapheresis CD34 cells/ $\mu\text{L}$	.066	.025	.260	2.602	.013	.015	.117
Interruption during LVL	−.328	.137	−.300	−2.397	.021	−.604	−.052

Dependant variable: stem cell yield ( $10^6$ ).**Table 5 – Adverse reactions and complications.**

During LVL (n = 55)		Post-ASCT (n = 55)	
Total no. of cases having complications = 39 (71%)		Pre-Engraftment	Post-Engraftment
Nausea/vomiting (28, 72%)	Hypocalcemia (7, 18%)	Nausea/Vomiting (51, 95%)	Neuropathy (7, 13%)
Fatigue (19, 49%)	Hypotension (6, 15%)	Mucositis (45, 82%)	Anemia (2, 4%)
Headache/light-headedness (19, 49%)	Catheter occlusion/leakage (3, 8%)	Fever (24, 44%)	SOS (2, 4%)
Bloating/abdominal distension (18, 46%)	Acute lung injury (2, 5%)	Diarrhea (21, 38%)	Thrombocytopenia (1, 2%)
Fever (16, 41%)	Hypomagnesemia (3, 8%)	Infections (15, 27%)	Leucopenia (1, 2%)
Bone pain (12, 31%)	Transient cytopenias (2, 5%)	Hyponatremia (4, 7%)	Lack of graft sustainability (2, 2%)
Skin rash (11, 28%)	Hypokalemia (2, 5%)	Hypokalemia (2, 4%)	
Diarrhea (9, 23%)	Citrate toxicity (1, 3%)		
Parasthesia (8, 21%)			

the LVL procedure (Table 5), which were of mild to moderate grade, and were managed successfully with standard procedures in all cases. Gastrointestinal disturbances (nausea and vomiting), mucositis, fever and infection were common complications encountered in the post-ASCT period (Table 5) and were successfully managed by following the standard treatment in all cases. We could not detect any difference in the incidence or severity of any ADRs during the LVL procedure or

post-ASCT period between these two groups (G-CSF vs. G-CSF plus Plerixafor).

## Discussion

This was a single-center prospective study on a uniform cohort of newly diagnosed multiple myeloma cases, from

whom we achieved a 100% success rate in harvesting the minimum target stem cell yield of  $2 \times 10^6$  CD34+ cells/kg in a single setting of LVL from peripheral blood. The baseline characteristics and the post-induction response status were evenly matched between the G-CSF and G-CSF plus Plerixafor groups. Generic formulation of G-CSF and Plerixafor are safe, well tolerated, effective and have no adverse effect on engraftment, nor on graft sustainability at D100 of the post-transplant. In addition, we have also categorically demonstrated that LVL is safe, cost-effective and has a minimum adverse effect, which can be managed by following standard protocols. This is corroborated by the cost-effectiveness results of other studies.<sup>12,13</sup> These findings have a significant importance in developing countries, in which the cost can be reduced to a great extent if the target cell yield is achieved in single apheresis procedure.

The success rate of the minimum yield of  $\geq 2 \times 10^6$  CD34+ cells/kg by using G-CSF and G-CSF plus Plerixafor have been reported by DiPersio et al. and Pusic et al. as follows: 47.3% vs. 86.7% and 81.2% vs. 81.4%, respectively.<sup>17,18</sup> The 100% success rate of achieving the primary endpoints in our study could be explained due to various factors, such as the following: all cases in the study were NDMM; efficient administration of induction CT with novel agents; achievement of deeper response (sCR/CR in 85% of the cases); pre-emptive uses of plerixafor when D4 CD34+ cell count was  $<10 \mu\text{L}^{-1}$ , and; judicious intervention of trained apheresis experts during the entire period of the apheresis procedure. The VTD regimen is one of the most efficient induction CTs, which has been considered as a standard protocol and was administered in all our cases. The median number of the induction CT was 5 (range 3–6), which correlates with a good depth of response in 85% of the cases (at the end of the induction CT). The intervention was made to match the color of the mononuclear cell collection (MNC) tube with the colorigram in all the cases of both groups. This fact also contributed to the high success rate of cell yield in this study.

The achievement of the high success rate of all the secondary efficacy endpoints is another important aspect of our study. The median neutrophil engraftment and platelet engraftment days were 12 and 16 and 12 and 15 in the G-CSF and G-CSF plus plerixafor groups, respectively, which are in accordance with other studies.<sup>17,19</sup> The D100 graft sustainability was very high in both the groups (95% and 97%, respectively). The ASCT has improved the depth of response in both the groups by achieving sCR in 90% and 100% of the cases, respectively. We could not ascertain the exact depth of response, as the MRD (minimal residual disease) was not measured due to the lack of such facilities at our institution. The target of  $\geq 5 \times 10^6$  CD34+ cells/kg was selected as one of the secondary endpoints to ensure the option of a possible tandem ASCT, in which two  $2 \times 10^6$  CD34+ cells/kg transplants could be possible. This factor has a relevance to institutions (including ours), in which a liquid nitrogen storage facility is available so that high risk myeloma patients or relapsed patients (two years after the 1st transplant) may be benefited from a tandem ASCT or 2nd ASCT, respectively.<sup>20–22</sup> This target was achieved in 66.7% of the cases in the G-CSF group and 61.7% of the G-

CSF plus Plerixafor group, which were quite satisfactory, in comparison with another study by John F. DiPersio et al., who achieved it in 34.4% of the G-CSF group and 71.6% of G-CSF plus Plerixafor group, by employing 2 or less conventional HPC(A) procedures.<sup>17</sup>

Different covariates, such as age, gender, BMI, BSA, Hb, leukocyte count, platelet count, Karnofsky PS, per-apheresis CD34 count and interruption during the LVL procedure, were studied with the stem cell yield in a Cox-regression analysis model. The age and number of interruptions during apheresis are two significant factors ( $p = 0.003$  and  $p = 0.021$ , respectively), which are inversely related to the stem cell yield, while the pre-apheresis CD34 count is another significant factor ( $p = 0.013$ ), which is directly proportionate to the stem cell yield. Other factors could not be considered to have a significant impact on the CD34+ yield. The pre-transplant depth of response has been correlated directly with the stem cell yield by other studies, but we failed to appreciate it. This could be due to the fact that the pre-transplant myeloma response was very similar in both groups, i.e., 100% sCR in the G-CSF + Plerixafor group and 90%, in G-CSF group. The age and pre-apheresis CD34 count have been significantly well correlated with the CD34 yield by various studies.<sup>23,24</sup> Judicious intervention and counseling/motivation of the patients to minimize the interruption during apheresis could be an important factor in achieving stem cell yield, a new finding revealed by the present study. This finding could be explained by the fact that the interface created by the apheresis machine is disturbed when the procedure is halted and the re-establishment of the interface takes time and the collection of stem cells is put on hold until the color of the MNC collecting tubing matches with the colorigram.

The generic molecules of both drugs used in the stem cell mobilization, i.e., G-CSF and Plerixafor are safe, well tolerated and effective, with very good engraftment and D100 depth of response/grant sustainability, which are comparable with other studies using the original molecules. The median duration of the LVL procedure was 330 minutes. Gastrointestinal disturbances, such as nausea/vomiting (28.72%), bloating and abdominal distension (18.46%) and fatigue (19.49%), headache/light-headedness (19.49%), transient fever (16.41%) and bone pain (12.31%) were common ADRs reported during the LVL procedure, while other events were reported in 3% to 18% of the cases (Table 5). All these adverse events were of mild to moderate severity and were managed by following the standard procedures. This suggests the safety of the LVL procedure, albeit with a long duration, vis-a-vis conventional HPC(A). Cytopenia (leukopenia and thrombocytopenia), gastrointestinal disturbances, mucositis, fever and infection were common ADRs during the post-ASCT period, which were consistent with the expected lines and in agreement with other studies (Table 5). All these events were managed by following standard care. The incidence, severity and successful management of ADRs were very similar in both groups. Two of our cases (out of the 34 in the G-CSF + Plerixafor group) developed myelodysplasia after D100 of the post-ASCT period, which explains the lack of graft sustainability. Its significance need be reascertained in larger groups in future studies.

## Conclusion

The LVL is safe, effective and can achieve the required minimum of CD34+ cells in a single apheresis, with minor adverse reactions, which can be easily managed by following the standard protocol. The number of interruptions during apheresis, pre-apheresis CD34 count and age are significant factors influencing the cell yield. Judicious intervention during the procedure, ensuring the quality of the CD34+ cell yield, by matching the colorigram may help in maintaining the adequate cell yield. All these factors are relevant to resource-constrained settings.

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## Conflicts of interest

The authors declare no conflicts of interest.

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