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Predictors of Poor Haematopoietic Stem Cell Mobilisation In Patients With Haematological Malignancies at a South African Centre

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Abstract

Background

Autologous stem cell transplant (ASCT) is an established consolidation strategy in the treatment of haematological malignancies, however poor mobilisation (PM) can contribute to patient morbidity and high resource utilisation. Identifying the incidence, risk factors for PM and engraftment outcomes are important goals in our resource limited setting.

Methods

We retrospectively analyzed patients with haematological malignancies that consecutively underwent ASCT at Groote Schuur hospital, Cape Town, South Africa from January 2013 to January 2019.

Results

146 patients – majority with multiple myeloma (MM)(41,8%), F:M=1:2, underwent leukapheresis with median age of 32 years (range, 9 – 66 years). PM occurred in 25/146 (17%), mobilisation failure (MF) in 3/146 (2%) and super mobilisation (SMs) in 99/146 (68%), respectively. Risk factors for PM were: diagnosis of acute leukaemia (RR=25, 95% CI 3.4 – 183, p=0.002) and Hodgkin lymphoma (RR=19, 95% CI 2.6 – 142, p=0.004); low white cell count (WCC) at harvest (WCC < $9 \times 10^9/L$ (RR=4.3, 95% CI 2.3 – 8.3, p < 0.0001) and two vs one line of prior therapy (RR=3.1, 95% CI 1.45 – 6.7, p=0.0037). Median days to neutrophil and platelet engraftment were 14 days (95% CI 14-15 days) and 16 days (95% CI 15-16 days) respectively.

Conclusion

PM occurred in 17% of a contemporary South African ASCT cohort, albeit with a low MF rate (2%). There was surprisingly high rate (68%) of SMs, possibly reflective of superfluous mobilisation strategy in MM patients. We identified predictive factors for PM that will lead to enhanced cost-effective use of plerixifor.

Keywords

Peripheral blood stem cells; poor mobilisation; leukapheresis; South-Africa

1. INTRODUCTION

Peripheral blood haematopoietic stem cells (PBSCs) are used to restore haematopoiesis more rapidly after high dose chemotherapy in treatment of multiple myeloma [1] (MM), non-Hodgkins lymphoma (NHL) [2], Hodgkins lymphoma (HL) [3] and good or intermediate-risk acute myeloid leukaemia (AML) [23], [30]. Mobilisation of haematopoietic stem cells (HSC) from the bone marrow to the peripheral blood can be performed by two methods: administration of granulocyte-colony stimulating factor (G-CSF) alone or by combining chemotherapy with G-CSF administration, both followed by leukapheresis [4]. It is well established that engraftment rates are influenced by the CD34+ stem cell dose infused and that a minimum dose of 2×10^6 CD34+ cells/kg has been shown to be safe for successful haematopoietic recovery [5], [6] – which in turn, has a direct impact on the duration of hospital stay and blood product utilisation. However, some patients fail to harvest an adequate amount of CD34+ cells after mobilisation [5], [7] (“poor mobilisation”[PM]) leading to a transplant delay or cancellation, and increased healthcare resource utilisation.

It is widely accepted that the peripheral blood CD34+ cell count (PBCD34) prior to apheresis is the most robust predictor of stem cell mobilisation [8], [9], however several studies have also identified some factors associated with PM which include: the amount of prior myelotoxic chemotherapy and radiotherapy [10], [11], [12], advanced age [10], [11], [13], diabetes mellitus [14], bone marrow involvement at diagnosis [5], [15] and thrombocytopenia [13], [16], [17]. Identifying these factors can possibly help predict which patients will be PM and therefore identify candidates for strategies such as the preemptive administration of plerixafor [18] - a molecule that inhibits the binding of chemokine receptor-4 with stromal-cell-derived factor-1, resulting in the enhanced release of CD34+ cells in the peripheral blood. Current guidelines recommends the preemptive use of plerixafor based on PBCD34 cell count monitoring, as it appears to prevent mobilization failure (MF) and may avoid unnecessary use of plerixafor [19], which is very expensive.

“Super mobilisers” (SM) are patients that mobilize more CD34+ cells ($> 8-10 \times 10^6/\text{kg}$) that is required for a single or tandem autologous haematopoietic stem cell transplant depending on the specific disease [20]. Infusing $> 8 \times 10^6/\text{kg}$ CD34+ cells and whether it has a positive impact on patient outcome is currently not known [21]. Therefore avoiding SM can also lead to more optimal use of the apheresis procedures and save costs.

At our high volume public transplant unit, identifying the burden of PM and SM, the risk factors that influence PM in our context and the engraftment kinetics, will lead to more optimal use of the costly apheresis procedure and plerixafor use – important goals in our resource limited setting. Our objective was to determine these factors, therefore we performed a retrospective review of all patients with haematological malignancies that underwent PBSC mobilisation and collection over a 7 year period.

2. MATERIALS AND METHODS

2.1. Patients

Between January 2013 and January 2019, 146 patients diagnosed with MM (risk unknown), 19 with NHL (4 Burkitt lymphoma, 6 diffuse large B cell lymphoma, 5 mantle cell lymphoma, 2 peripheral T-cell lymphoma, 1 plasmablastic lymphoma and 1 primary CNS lymphoma), and 32 with HL. Thirty-three patients had acute leukaemia (AL)/lymphoblastic lymphoma (LL) (14 favorable risk acute myeloid leukaemia (AML) – according to ELN classification [22]; 9 intermediate risk AMLs without a suitable allogeneic donor, 3 acute promyelocytic leukaemias (APL) in 2nd CR and 7 T-cell LLs planned for autologous haematopoietic stem cell transplant (ASCT) underwent PBSC mobilisation and leukapheresis in the haematology apheresis unit at Groote Schuur hospital (GSH) affiliated

to the University of Cape Town, Cape Town, South Africa. For the patients with favorable and intermediate risk AML without a sibling donor available, as per our local protocol, we consolidate with ASCT as we (Novitsky et al.) have previously shown comparable survival rates between recipients of allogeneic versus autologous stem cell transplant. Written informed consent for the PBSC procedure and data collection was obtained from each donor prior to commencement of the procedure. Medical records of all patients (for baseline characteristics and apheresis information) were collected retrospectively for analysis. This retrospective cohort study was approved by the University of Cape Town Human Research Ethics Committee (HREC number 078/2017).

2.2. Mobilisation procedure and collection

All patients planned for ASCT followed the GSH Haematology unit protocol for the mobilisation and collection of PBSCs. Donors received Etoposide 1000 mg/m² IV for two consecutive days (Day 1 and 2 of the protocol) in the outpatient department. G-CSF was commenced at 10ug/kg daily subcutaneously from day 5 up to day 15; a split dose regimen of 300ug twice daily (300ug Filgrastim vials were used). Patients would be followed up on days 9 and 11 to check their blood counts and renal function, assess the patient clinically and to ensure compliance with G-CSF administration. On day 14 and 15 a PBCD34 count was obtained to assess the likelihood of a successful CD34 collection. Patients with a PBCD34 < 10 cells/uL were continued on G-CSF for up to four days depending on the PBCD34 and WCC response. If the PBCD34 reached a plateau despite ongoing G-CSF administration, apheresis was abandoned. Apheresis was performed utilising a continuous flow apheresis system (Spectra Optia, Terumo BCT) through a percutaneous femoral venous catheter.

2.3. Enumeration of peripheral blood CD34+ cells

Multiparametric flowcytometry using monoclonal antibodies CD45 (FITC, IOTest, Beckman-Coulter) and CD34 (PE, IOTest, Beckman-Coulter) was performed to immunophenotype circulating CD34+ cells in the peripheral blood. 200 000 cells were acquired for analysis using a Fc500 flowcytometer (Beckman-Coulter) and CXP software (Beckman-Coulter). The ISHAGE protocol was followed to determine the CD34+ population of cells while dual platform analysis was utilised for CD34+ cell quantification.

2.4. Definition of poor mobilisers (PM)

Patients that achieved a PBCD34 count of < 20 cells/ul at maximal stimulation were defined as PMs. Furthermore, PMs were subdivided: patients with PBCD34 counts between 11 to 19 cells/ul were defined as "borderline PMs", patients with counts between 6 to 10 cells/ul

were defined as “relative PMs” and patients with a PBCD34 < 5 cells/ul were defined as “absolute PMs” [12].

2.5. Definition of super-mobilisers (SM) [31]

Patients in whom a total of $>8 \times 10^6$ /kg CD34+ cells was collected in the final apheresis bag.

2.6. Definition of neutrophil and platelet engraftment [26]

Neutrophil engraftment is defined as the first date when absolute neutrophil count (ANC) of $\geq 0.5 \times 10^9$ /L was achieved for three consecutive days. Platelet engraftment was defined as the first date of three consecutive days that platelets are $\geq 20 \times 10^9$ /L without platelet transfusions in the last seven consecutive days.

2.7. Data analysis

The association between the PBCD34 count and CD34 yield in the apheresis product was evaluated by the Spearman rank correlation. Donor characteristics included in the binomial regression analysis were: age, gender, weight, body mass index (BMI), haematological diagnosis, pre-mobilisation blood cell parameters i.e. haemoglobin, platelet count, absolute neutrophil count, absolute monocyte count, absolute lymphocyte count and total white cell counts. In addition, the mid-mobilisation (day 9 of mobilisation) blood cell parameters and blood cell parameters on day of apheresis and previous lines of chemotherapy were also analysed. Age and weight were categorised into approximate quartiles. The relative risk (RR) of each variable on PM was determined using binomial regression. Categories with N<15 were not included in analyses as no reliable inference can be made based on such small groups. Donor characteristics/variable significant at $p<0.15$ were combined into a multivariable model, after examining each pair of variables for possible confounding using the chi-square test (or Fisher’s exact test for 2x2 tables). A value of Cramer’s V (or the phi coefficient for Fisher’s exact test) > 0.50 was regarded as too strong an association to include both variables in a multivariable model. Non-significant variables were sequentially removed from the multivariable model. The association between PM and final CD34 harvest, was determined by the Wilcoxon Rank Sum test (since the data did not meet the assumptions of the independent samples t-test) and Fisher’s exact test, respectively. The association between PM and days to neutrophil engraftment and days to platelet engraftment was determined by Cox Proportional Hazards regression, with censoring for death. Statistical analysis was carried out using SAS version 9.4 for Windows. A 0.05 p-value for significance level was used. Graphs were created with the software GraphPad Prism Version 8.0.2. and Excel.

3. RESULTS

3.1. Patient characteristics

Patient and disease characteristics are summarised in [Table 1](#). Between January 2013 and January 2019, 146 consecutive patients were scheduled to undergo leukapheresis for ASCT: median patient age was 45 years (range, 9 - 66 years), the majority (61%) were male and (67.8%) received only one previous line of therapy. Disease groups that underwent ASCT were MM (41.8%), lymphoma (39.7%) and AL (18.5%). The majority of MM patients (41.8%) achieved a VGPR before transplant, while 28.1% were in CR1 and 30.1% in > CR1. HIV infection affected 3.4% of the entire cohort. The majority (56.8%) had not received radiotherapy prior to ASCT.

Table 1. Patient Characteristics.

Variable	Category	n	(%)
Age	9-29 y	40	(27.4)
	30-45 y	35	(24.0)
	46-55 y	45	(30.8)
	56 y+	26	(17.8)
Gender	F	57	(39)
	M	89	(61)
Diagnosis	MM	61	(41.8)
	HL/NHL/T-LL	58	(39.7)
	AML	27	(18.5)
Lines of therapy	1	99	(67.8)
	2	26	(17.8)
	>3	21	(14.4)
Previous radiotherapy	Yes	7	(4.8)
	No	83	(56.8)

	Missing	56	(38.4)
HIV infection	Yes	5	(3.4)
	No	141	(96.6)
Disease status at SCT	CR1	41	(28.1)
	> CR1	44	(30.1)
	VGPR	61	(41.8)
Blood cell parameters on collection day		Median	Range
WBC x 10⁹/L		24	(2.83 – 106.7)
Neutrophils x 10⁹/L		14.9	(0.00 – 69.6)
Lymphocytes x 10⁹/L		1.94	(0.39 – 20.27)
Monocytes x 10⁹/L		2.11	(0.01 – 28.74)
Haemoglobin, g/dL		9.55	(7.00 – 15.2)
Platelets x 10⁹/L		94	(16.00 - 339)

Abbreviations: F, female; M, male; MM, multiple myeloma; HL, Hodgkins lymphoma; NHL, non-Hodgkins lymphoma; T-LL, T-lymphoblastic lymphoma; AML, acute myeloid leukaemia (includes 3 APLs) CR, complete remission; VGPR, very good partial response; HIV, human immunodeficiency virus.

3.2. Utility of peripheral blood CD34 (PBCD34) to predict CD34 yield

Fig. 1 A illustrates a significantly good correlation between the PBCD34 count on day of apheresis and the amount of CD34+ cells in the final apheresis bag. **Fig. 1B** shows that patients who achieved a PBCD34 count ≥ 20 cells/uL at maximal stimulation could reliably predict a single day apheresis in our cohort.

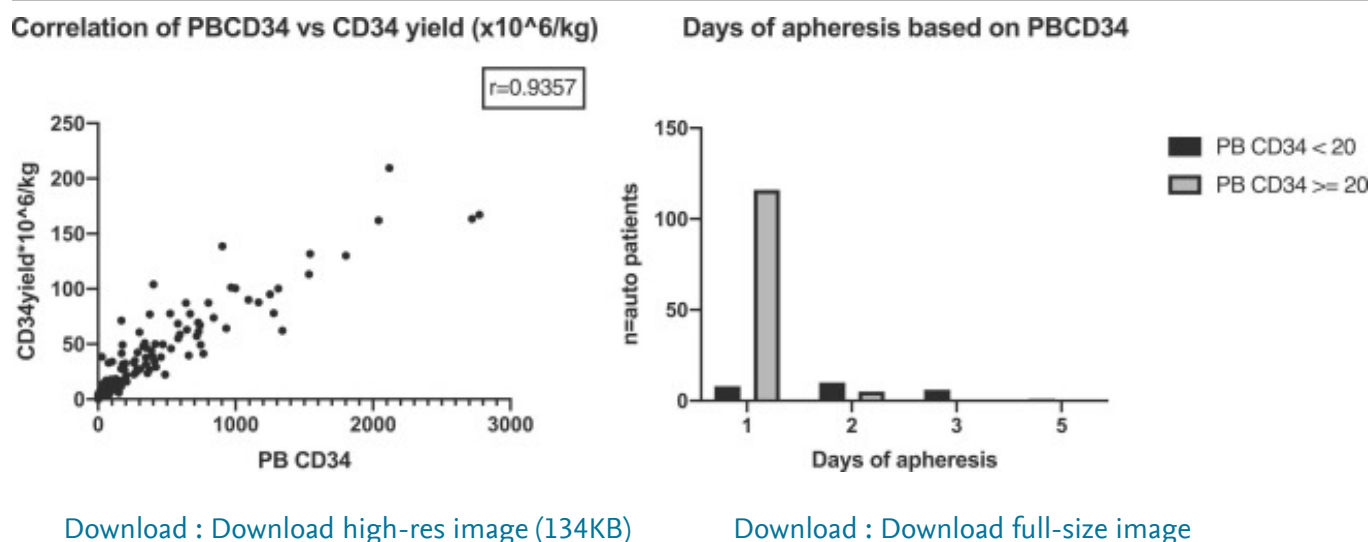


Fig. 1. A. Correlation between the PBCD34 counts (on day of apheresis) and final CD34 yield. Fig. 1 B. Days of apheresis according to PBCD34 counts.

3.3. Incidence and clinical characteristics of PMs

Twenty-five patients were PMs (17.1%): 14 (9.6%) absolute PMs, 2 (1.4%) relatively PMs and 9 (6.2%) borderline PMs. The absolute and relative PMs were young patients with median ages of 27 (range, 16-58) and 39 years (range, 27-51), respectively. For both the absolute and relative PMs, the median previous lines of therapy were 2 (range, 1-3). The borderline PMs had a median age of 32 (range, 19-53) and a median of 1 line of therapy prior to ASCT. In all three groups of PMs, the majority were females with the diagnoses of HL and AML. See [Table 2](#).

Table 2. Incidence and clinical characteristics of the PMs.

Poor mobilisers/Diagnosis	N=25 (17%)	Days of apheresis (Med,range)	Minimum target (2 ×10 ⁶ /kg CD34) achieved	Sex	Age (med,range)	Prev lines of therapy (med,range)
Absolute PM (<5 PBCD34 cells/ul)	n=14(9.6)	2 (1-5)	Yes=11 No=3	F=10 M=4	27 (16-58)	2 (1-3)
- HL=6						

- AML=5
- NHL=2
- MM=1

Relative PM (6-10 PBCD34 cells/ul) n=2(1.4) 2 Yes=2 F=1 39 (27-51) 2 (2-3)

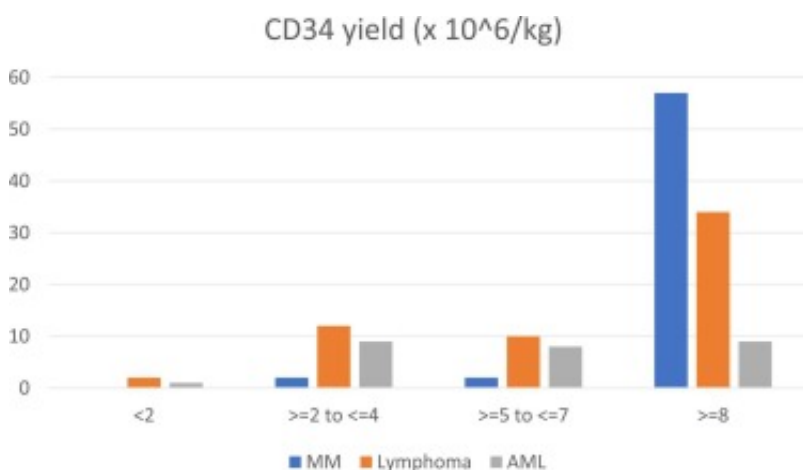
- AML=1 No=0 M=1
- HL=1

Borderline PM (11-19 PBCD34 cells/ul) n=9(6.2) 1 (1-3) Yes=9 F=4 32 (19-53) 1 (1-3)

- AML=5 No=0 M=5
- HL=3
- NHL=1

3.4. Spread of super-mobilisers

68% of patients experienced a high CD34 yield (≥ 8 CD34 cells $\times 10^6$ /kg) in the final apheresis product (“super-mobilisers”). The majority of these patients had multiple myeloma (57%), while 34% had lymphoma (both NHL and HL) and 9% had AML (Fig. 2).



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Fig. 2. Proportions of the final apheresis yield (CD34+ cells x 10⁶/kg) of entire series.

3.5. Univariate analysis

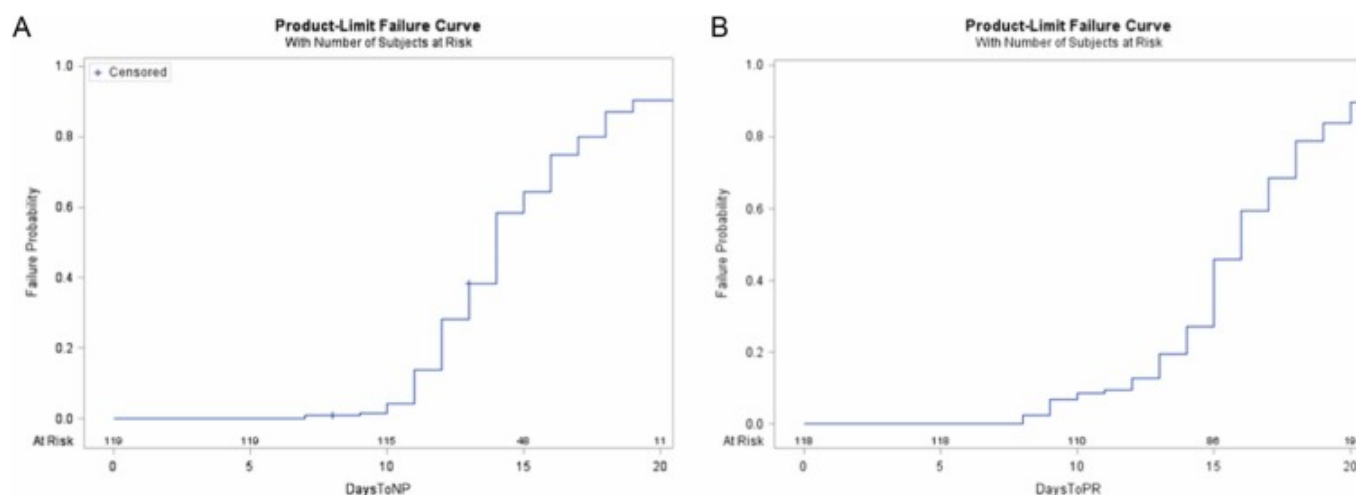
The incidence of PM was associated with a diagnosis of AL (AL vs. MM, RR=25, 95%CI 3.4 - 183) and HL (HL vs. MM,RR=19, 95% CI 2.6 – 142). On day of collection, PM was associated with a lower platelet count (platelets < 90 vs. platelets \geq 90, RR=8.9, 95% CI 2.8-28), lower absolute neutrophil count (< 7.5 vs. \geq 7.5, RR=2.7, 95% CI 1.35 – 5.4), lower monocyte count (< 0.6 vs. \geq 0.6, RR=2.4, 95% CI 1.05 - 5.3) and lower total white cell count (< 9 vs. \geq 9, RR=4.3, 95% CI 2.3 – 8.3). PM was significantly associated with 2 lines of therapy vs. one line (RR=3.1, 95% CI 1.45 – 6.7). No significant differences were found for baseline and mid harvest blood cell parameters.

3.6. Multivariable analysis

In addition to the variables identified in the univariate analysis, age, mid-harvest haemoglobin, mid-harvest platelet count, mid-harvest absolute monocyte count and the at harvest haemoglobin were significant at $p < 0.15$ and were thus candidate variables for a multivariable model for PM. Before embarking on the multivariable model, we checked pairs of candidate variables for possible confounding. 11 variables and 16 parameters were to be estimated; since sample size considerations dictate that $n=25$ cases with PM allowed for the estimation of only 2 parameters. Therefore, we could not fit a multivariable model and instead we considered the effects of each of the significant variables as shown in the univariate analysis.

3.7. Cumulative incidence of neutrophil and platelet engraftment

The median number of days to neutrophil engraftment was 14 days (range, 4 - 34 days) whereas the median number of days to platelet recovery was 16 days (range, 8 - 54 days). There were no significant differences in neutrophil ($p=0.15$) and platelet engraftment ($p=0.70$) when comparing PM with non-PMs. (Fig. 3)



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Fig. 3. A. Cumulative incidence of neutrophil engraftment and 3B platelet engraftment.

4. DISCUSSION

In this cohort of patients, we observed a PM rate of 17%, a low rate of MF (2%) and a high rate of super-mobilisers (68%). A diagnosis of AL and HL with low blood cell parameters on day of harvest were identified as risk factors for PM.

We show a surprisingly high rate of super-mobilisers in this cohort (68%), which predominantly consisted of MM patients (57%). This can be explained by the exclusion of thalidomide or lenalidomide in our remission induction therapy and the use of high dose etoposide ($1000 \text{ mg/m}^2 \times 2 \text{ days}$) during CM – this implies an over-effective CM strategy. We should be mindful that high dose etoposide can lead to harmful side-effects like mucositis, hepatic dysfunction and prolonged cytopenias with subsequent life-threatening complications like neutropenic sepsis. Song et al. has previously shown that a lower dose etoposide of 375 mg/m^2 during CM for MM to be safe and effective [23]. Therefore, the use of lower dose etoposide should be explored for our MM patients while the higher dose etoposide is appropriate for the lymphoma and AL disease groups.

We confirm the utility of the PBCD34 counts in our cohort, as there is a good correlation between the PBCD34 and CD34 yield. Additionally, if a patient achieved a PBCD34 count $> 20 \text{ cells/ul}$ at maximal stimulation with CM that they will mostly harvest successfully in a single day – an important goal in our context.

On univariate analysis we identified that a diagnosis of AL and HL to be associated with PM. As per our local protocol, favourable and intermediate risk AML patients (without suitably matched HLA donors) receive two cycles of “7+3” chemotherapy (7 days cytarabine with 3 days of daunorubicin) followed by ASCT, as we have previously shown similar outcomes in both autologous and allogeneic stem cell transplant recipients in this context [24]. Shin et al. has previously shown that the stem cell yield linearly decreases after each consolidation chemotherapy in patients with AML [25] and we confirm this observation. For the patients with HL, the majority had advanced stage disease at diagnosis and were heavily pre-treated prior to referral for ASCT. Both factors are known to be associated with PM [10], [11], [12].

Other risk factors identified on univariate analysis were low blood cell parameters at harvest – specifically total white cell count (WCC) < 9, absolute neutrophil count < 7.5, monocyte count < 0.6 and platelet count < 90. This might reflect poor stem cell reserve prior to start of CM leading to a delayed recovery, possibly bone marrow involvement at diagnosis and/or non-compliance with G-CSF during mobilisation. It has previously been shown that there is a poor correlation between total WCC and CD34 yields, making it a less reliable marker to assess start of apheresis [26], [27]. If the total WCC is low, the PBCD34 is likely to be low leading some to suggest that PBCD34 should be measured when the WCC exceeds a certain threshold [26], [28], [29]. Our data show that the probability of achieving an acceptable PBCD34 for successful mobilisation is only achieved when WCC is >9. This will lead to fewer unnecessary PBCD34 measurements at lower WCCs and save costs.

The 3 patients that failed harvest (2%) in this series could arguably have benefited from “on demand” plerixafor [18]. Based on the current data we can now identify patients at high risk of MF for which we can apply for its use, specifically, for patients with AL or HL with PBCD34 counts at maximal stimulation of <5 cells/ul. This will lead to more efficient use of this expensive drug while preventing patient morbidity.

The engraftment rates for both neutrophils and platelets were good with no significant differences found between the PM and non-PMs. Therefore, we confirm that patients receiving a minimal CD34 dose of 2×10^6 /kg engraft well in our cohort.

Limitations of our study include its retrospective nature and heterogenous sample. However, the homogeneity of the clinical staff in our transplant unit, CM regimen, uniformity of our CD34 apheresis sessions (using the same cell separator) and CD34 enumeration adds additional value. This is the first retrospective study done on stem cell mobilisation from the only public sector teaching hospital with its own designated apheresis unit in South Africa.

In conclusion, we describe a PM rate of 17%, a low MF rate of 2% and a surprisingly high rate of super-mobilisers (68%) reflecting an over-effective CM strategy, especially in MM patients. We confirm the utility of the PBCD34 count and a target of > 20 cells/ul post CM will mostly lead to a single day apheresis. Finally, we identified risk factors for PM that will lead to more rational use of plerixafor.

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CRedit Author statement

JDT contributed to the conceptualising, planning, data capturing, writing and editing of the article. EV and MS assisted with writing, reviewing and editing of the article.

Declaration of competing interests

None.

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