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Analysis of CD34⁺ cell collection using two mobilization regimens for newly diagnosed multiple myeloma patients reveals the separate impact of mobilization and collection variables

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Abstract

Mobilization regimens for CD34⁺ cells have generally been judged successful based on the number of cells collected without evaluating mobilization separately from collection. Using retrospective data for patients who collected CD34⁺ cells on Total Therapy protocols 3a/3b (VTD-PACE) and Total Therapy 4/5 using a novel regimen that added low dose melphalan to VTD-PACE (MVTD-PACE), we analyzed mobilization and collection variables separately. A significant difference favoring MVDT-PACE was found in mean CD34⁺ cells/ μ L on day 2 of collection and in mean ratio of CD34⁺ cells/ μ L on day 2 to day 1. However, because apheresis variables and growth factor dose during collection were manipulated to optimize individual collections, the two regimens were not significantly different when the mean total CD34⁺ cells $\times 10^6$ /kg collected was compared. Thus, when evaluating a chemotherapy regimen or new growth factor for mobilization it is important to realize that total CD34⁺ cells collected is dependent on both mobilization and collection variables.

Keywords

CD34⁺; mobilization; apheresis; myeloma; melphalan

INTRODUCTION

The optimal mobilization strategy for collecting CD34⁺ hematopoietic progenitor cells (HPC) to support autologous transplantation for patients with multiple myeloma (MM) remains undefined [1], because collection goals vary among centers [2]. Most centers consider a CD34⁺ cell dose between 2 and 5 $\times 10^6$ /kg an acceptable transplant dose and therefore collection goal [3–6]. Collection has been tacitly assumed to be a simple reflection

of mobilization by many workers, thus for most centers a mobilization regimen which yields a collection of at least 2×10^6 CD34⁺ cells/kg has been considered to be a good regimen.

However, the goal at our institution has been for many years to obtain at least 20×10^6 CD34⁺ cells/kg so that salvage interventions can be accommodated post-transplant. It has therefore been important to look more closely at what constitutes a good mobilizing regimen, and whether collection is a simple reflection of mobilization. Here we analyze separately mobilization and collection of HPC using our previous regimen, VTD-PACE, in Total Therapy 3a and 3b, and compare it to the current regimen MVTD-PACE, employed in Total Therapy 4 and 5, in order to see if this assumption is valid.

MATERIALS AND METHODS

Patient Population

This is a single institution retrospective data review for all patients who collected HPC on Total Therapy (TT) protocols with VTD-PACE or MVTD-PACE at the Myeloma Institute for Research and Therapy, University of Arkansas for Medical Sciences, Little Rock, AR. All patients were recently diagnosed with MM, and signed research consents approved by the Institutional Review Board. Patient characteristics are shown in Table I. One patient was excluded from the VTD-PACE arm because data for the number of CD34⁺ cells/ μ L on the second day of collection was unavailable.

MOBILIZATION

The two regimens compared here are VTD-PACE in TT3a/TT3b (bortezomib, thalidomide, dexamethasone, cisplatin, doxorubicin, etoposide, cyclophosphamide) [7,8] with data collected from February 2004 to September 2008; and MVTD-PACE in TT4/TT5 (melphalan, bortezomib, thalidomide, dexamethasone, cisplatin, doxorubicin, etoposide, cyclophosphamide) [9,10] with data collected from August 2008 to May 2011. TT3a (2003–33) and TT3b (2006–66) enrolled newly diagnosed patients with up to 1 cycle of prior therapy with different maintenance regimes. Patients on TT3a/b were used to develop a high risk/low risk gene expression profile (GEP) based risk score [7,8]. TT4 (2008–01) then enrolled low risk patients defined by a 70-gene derived GEP based risk score <0.66 , no TP53 deletion (Affymetrix signal <727), no metaphase based abnormalities of 1q or 1p or LDH <360 IU/L while TT5 (2008–22) enrolled high risk patients defined by a 70-gene derived GEP based risk score ≥ 0.66 , the presence of TP53 deletion (Affymetrix signal ≥ 727), metaphase based abnormalities of 1q or 1p or LDH ≥ 360 IU/L. A small melphalan test-dose (10mg/m^2) was added to VTD-PACE in TT4/5 to allow pharmacogenomics analysis, leading to the development of an additional prognostic GEP-80 model [11]. The timing of other drugs relative to the small dose of Melphalan was shifted by 24 hours between TT3a/TT3b and TT4, and the dose of Bortezomib with TT5 was slightly greater than TT3a/TT3b and TT4. Both mobilization regimens called for the subcutaneous administration of filgrastim (G-CSF, $5\mu\text{g/kg}$) twice a day for a total dose of $10\mu\text{g/kg}$ daily. We did not collect data on number of days of G-CSF given prior to starting collection, as a patient first ready for collection on a given day might have been delayed depending on when the line placement was able to be scheduled. G-CSF doses were adjusted upwards to 8

μg/kg twice a day if the predicted collection was inadequate to achieve the collection goal. Details of the two regimens are shown in Table II.

HPC Collection and Flow Cytometry

The peripheral blood absolute CD34⁺ cell count was monitored daily in the morning using ProCount™ (Becton-Dickinson, San Jose, CA, USA), beginning when the white blood cell count recovered to 2000/μL after chemotherapy. As this test is only done Monday-Friday at our institution, testing was not always started immediately the white blood cell count was appropriate to the test. Apheresis was initiated using a central venous catheter placed by interventional radiology in the internal jugular position when the predicted CD34⁺ cell collection for 30L of blood processed (large volume leukapheresis, LVL) was at least 1×10⁶/kg using our center's predictive formula [12]. The volume of blood processed each day was based on our predictive formula [12], and ranged from 5 – 30L. Cells were collected on a COBE® Spectra apheresis machine (Terumo®, Lakewood, Colorado, USA) using 1,000 mL ACD and 5,000 units heparin for anticoagulation at an inlet:anticoagulant ratio of 31:1, and an inlet flow rate of 150 mL/min with anticoagulant infused at 5 mL/min. Collection flow rate was set at 1.5 mL/min and 10 mL of ACD was added to the component at processed volumes of 10 L, 20 L, and 30 L. An infusion of 2 g calcium chloride in 250 ml normal saline (0.9% sodium chloride) ran at 85 ml/h.

Apheresis was discontinued when patients collected at least the minimum number of CD34⁺ cells required by their treatment protocol (minimum 2 day collection required per protocol).

For data analysis, patients were stratified into 4 mobilizer types based on the predicted first day CD 34⁺ cell collection with LVL of 30L: poor (<2×10⁶ CD34⁺ cells/kg), intermediate (2 to 10×10⁶/kg), good (>10 to 20×10⁶/kg) and excellent (>20×10⁶/kg). CD34⁺ cell enumeration in the products collected was done by flow cytometry on a FACSCalibur (Becton-Dickinson) using the ISHAGE protocol [13].

Statistical Analysis

The variables examined included peripheral blood CD34⁺ cells/μL on day 1 and day 2 of collection, the ratio of CD34⁺ cells/μL on day 2 of collection to day 1, total CD34⁺ cells ×10⁶/kg collected, total liters processed and number of collection days. These variables were examined in subgroup analysis for gender (male vs. female), age (<65 vs. ≥65), race (Caucasian vs. African American/Hispanic/other), and mobilizer types (poor, intermediate, good, and excellent). The ratio of CD34⁺ cells/μL on day 2 of collection to day 1 was calculated to account for the large measure of dispersion between the 2 regimens. The means of peripheral blood CD34⁺ cells/μL on day 1 and day 2 of collection, the ratio of peripheral blood CD34⁺ cells/μL on day 2 of collection to day 1, total CD34⁺ cells ×10⁶/kg and their subgroups were compared using two-tailed student's t-test.

RESULTS

The results of our statistical analysis for mobilization and collection are presented separately below, and are summarized in Table III.

Mobilization

A significant difference in mobilization was seen when the mean number of CD34⁺ cells/ μ L on day 2 of collection was compared between VTD-PACE and MVTD-PACE (219.9 vs. 294.3; $P = .001$). Significant differences were seen in subgroup analysis for the two regimens for the following variables: male gender (210.8 vs. 300.8; $P = .002$), <65 (227.7 vs. 318.2; $P = .001$), Caucasian (221.6 vs. 295.5; $P = .001$). Significant differences were also found when the mean ratio of peripheral blood CD34⁺ cells/ μ L on day 2 of collection to day 1 was compared between VTD-PACE and MVTD-PACE (1.19 vs. 1.46; $P = 0.001$). In subgroup analysis this statistical significance was also found for female gender (1.09 vs. 1.52; $P = 0.001$), <65 (1.20 vs. 1.50; $P = 0.001$), Caucasians (1.19 vs. 1.42; $P = 0.003$), intermediate mobilizers (1.36 vs. 1.82; $P = .007$), and good mobilizers (1.21 vs. 1.52; $P = 0.03$).

Collection

The average total liters of blood processed for collection was higher with VTD-PACE than with MVTD-PACE (23.2 vs. 20.5L), both overall and for all mobilizer subgroups, and the mean number of collection days was slightly higher (2.4 vs. 2.3 days). The mean total CD34⁺ cells $\times 10^6$ /kg collected for all patients was slightly lower with VTD-PACE than MVTD-PACE but was not statistically significant except in subgroup analysis for male gender (33.3 vs. 37.5; $P = .04$) and intermediate mobilizers (23.7 vs. 26.3; $P = .03$).

There were 5 patients (71%) in the VTD-PACE poor mobilizer group who required an increase in G-CSF dose from 10 μ cg/kg/day to 16 μ cg/kg/day and 2 patients (50%) in the MVTD-PACE poor mobilizer group who required an increase. However, there were too few poor mobilizers for statistical analysis.

DISCUSSION

Here we use circulating CD34⁺ cells in the peripheral blood as a measure of mobilization to evaluate VTD-PACE compared to MVTD-PACE. Because it provides a higher peak number of CD34⁺ cells/ μ L in the blood, as demonstrated by the ratio of CD34⁺ cells/ μ L on day 2 of collection to day 1, MVTD-PACE is a better mobilizing regimen than VTD-PACE. Further, for each of the variables found on subgroup analysis of mobilization to be statistically significant, MVTD-PACE was better than VTD-PACE. The fact that some subgroups had a significant improvement in mobilization and others did not may reflect the difference in mobilizing capacity previously reported in normal donors for race, sex, age, total dose of G-CSF given and body mass index [14].

Melphalan has only rarely been used as part of a mobilization regimen meant to allow HPC collection [15–17], and neither data on daily CD34⁺ cells/ μ L nor information on whether G-CSF dose was adjusted is included in previous reports. The reason why addition of this small dose of melphalan to VTD-PACE is associated with improved mobilization needs further investigation, but may derive from the vigorous rebound out of cytopenia seen even in the earliest studies of this drug [18]. Nevertheless, despite the improved mobilization seen with MVTD-PACE, the total number of CD34⁺ cells collected did not differ significantly

between the two regimens as a whole because variables such as dose of G-CSF during collection and number of liters of blood processed daily were manipulated in order to optimize collection for individual patients. It is common for clinicians to adjust growth factors and apheresis to optimize collection, but analysis of two closely similar mobilization regimens has yielded an unusual opportunity to look at the influence of collection variables relative to mobilization on total number of HPC collected.

Conclusions

Mobilization of CD34⁺ cells into the blood alone does not determine what the total number of CD34⁺ cells collected will be. CD34⁺ cell collections can be optimized for individual patients by using a predictive formula to guide growth factor dose and volume of blood processed during apheresis. When comparing mobilization regimens it is therefore important to analyze mobilization of cells into the blood separately from the total number of cells collected before deciding that one mobilization regimen is better than another. Equally, when evaluating a published report in the literature regarding mobilization it is important to see whether information on the trigger for starting apheresis collections and data on mobilization of cells into the blood are presented, or only data on the number of cells collected.

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Table 1

Patient Characteristics and Mobilization Regimens

	VTD-PACE	MVTD-PACE
Total Number of Patients	393	188
Gender (%)		
Males	256 (65.1)	110 (58.6)
Females	137 (34.9)	78 (41.4)
Median Age (Range)	59.3 (30.7–74.7)	58.5 (30.4–75.9)
Younger than 65 year old (%)	289 (73.5)	133 (71)
Older than 65 year old (%)	104 (26.5)	55 (29)
Race (%)		
Caucasians	365 (92.8)	164 (87.3)
African-Americans	19 (4.8)	20 (10.7)
Hispanics	5 (1.3)	1 (0.5)
Others	4 (1.1)	3 (1.5)
Mobilizer Groups (%)		
Excellent	184 (46.8)	92 (48.9)
Good	102 (25.9)	40 (21.3)
Intermediate	100 (25.4)	52 (27.7)
Poor	7 (1.9)	4 (2.1)

Table II

Details of Mobilization Regimens

	TT3a/TT3b Protocols*	TT4/TT5** Protocols
Induction Regimen	Bortezomib 1mg/m ² (SC) Day 1,4,8,11 Thalidomide 200 mg (PO) Day 4-7 Dexamethasone 40 mg (PO) Day 4-7 Cisplatin 10 mg/m ² (CI) Day 4-7 Adriamycin 10 mg/m ² (CI) Day 4-7 Cyclophosphamide 400 mg/m ² (CI) Day 4-7 Etoposide 40 mg/m ² (CI) Day 4-7	Melphalan 10 mg/m ² (IV) Day 3/Day 1 Bortezomib 1 mg/m ² (IV) Day 1,5,8,11/ 1.3 mg/m² Day 4 and 7 Thalidomide 200 mg (PO) Day 5-8/Day 4-7 Dexamethasone 40 mg (PO) Day 5-8/Day 4-7 Cisplatin 10 mg/m ² (CI) Day 5-8/Day 4-7 Adriamycin 10 mg/m ² (CI) Day 5-8/Day 4-7 Cyclophosphamide 400mg/m ² (CI) Day 5-8/Day 4-7 Etoposide 40 mg/m ² (CI) Day 5-8/Day 4-7
G-CSF Dose (2 divided doses) Timing	10 µg/kg/day Starts on day after completion of chemotherapy	10 µg/kg/day Starts on day after completion of chemotherapy When WBC count <1500/µL
CD34+ Cells/µL	Starts when WBC count is 2000/µL	Starts when WBC count is 2000/µL
Target CD34+ Cells/kg ×10 ⁶	20×10 ⁶ CD34+ Cells/kg	20/ 25 ×10 ⁶ CD34+ Cells/kg
Criteria to Start Leukapheresis	Predicted CD34+ cell count at 30L blood processed 1×10 ⁶ /kg	Predicted CD34+ cell count at 30L blood processed 1×10 ⁶ /kg

IV: Intravenous, PO: orally, CI: Continuous Infusion, G-CSF: Granulocyte-Colony Stimulating Factor, WBC: White Blood Cell, SC: Subcutaneous

* The induction regimen for TT3a and TT3b protocols is identical in terms of dosing

** Bold indicates TT5 details in the induction chemotherapy regimen

Table III

Analysis of Variables Examined

Variable (all patients)	VTD-PACE	MVTD-PACE	P
Mean CD34+ Cells/ μ L on Day 1 of Collection	224.2	255.8	0.17
Mean CD34+ Cells/ μ L on Day 2 of Collection	219.9	294.3	0.001
Males	210.8	300.8	0.002
Younger than 65 year old	227.7	318.2	0.001
Caucasians	221.6	295.5	0.001
Mean Ratio of CD34+ Cells/ μ L on Day 2 of Collection to Day 1	1.19	1.46	0.001
Females	1.09	1.52	0.001
Younger than 65 year old	1.20	1.50	0.001
Caucasians	1.19	1.42	0.003
Good mobilizers	1.21	1.52	0.03
Intermediate mobilizers	1.36	1.82	0.007
Mean Total CD34+ Cells/kg $\times 10^6$ Collected	34.9	37	0.14
Males	33.3	37.5	0.04
Intermediate mobilizers	23.7	26.3	0.03
Average Total Liters Processed (Range)	23.2 (5–40)	20.5 (5–40)	N/A
Mean/Median Number of Collection Days (Range)	2.4/2 (2–9)	2.3/2 (2–10)	N/A

N/A Not Applicable

Bolded *P* values indicate statistical significance