

Prediction of CD34+ PBSC Collection: G-CSF and chemo mobilisation versus G-CSF mobilisation alone - is the Peripheral Blood CD34 always reliable?

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INTRODUCTION:

Despite its acceptance as an effective treatment option in a range of haematological & other settings, peripheral blood stem cell (PBSC) collection & cryopreservation for later re-infusion post-myeloablative therapy remains a highly resource consumptive procedure¹. The role of peripheral blood (PB) CD34 counts in predicting optimal timing of PBSC collection is well established and contributes to a more efficient means of obtaining adequate CD34 dose (minimum 2x10⁶/kg at our centre). A range of factors contribute to PBSC collection efficiency including optimal apheresis performance, patient body weight, disease and mobilisation regimen to name a few. Although most centres around the world use the PB CD34 count to predict collection CD34 dose, occasional discordant results occur despite patient body weight and collection volume being factored into result calculation.

AIM:

Our primary aim was to investigate the affect that mobilisation regimen had on the ability of the PB CD34 count (x10⁶/L) to predict the collection CD34 (x10⁶/kg). Additionally, we were interested in the affect other important variables had on this predictive power, namely patient body weight and PB CD34 range.

METHODS:

We performed a retrospective analysis on 519 consecutive PBSC collections (179 patients) during the recent 24 month period.

Autologous PBSC collections were performed by the apheresis unit using the Cobe Spectra apheresis machine programmed to process 2x blood volumes based on entry of appropriate patient data.

Collections that involved an apheresis complication that led to decreased or prolonged apheresis process volume were removed from the analysis (n=25).

Viable PB CD34 & collection CD34 counts were performed using a single platform method on a BD FACSCalibur™ with ISHAGE gating strategy². Briefly, a volume of whole blood or buffy coat were incubated with 10ul of CD45 FITC (Beckman Coulter clone J33), 10ul of CD34 PE (Beckman Coulter clone 581) and 10ul of 7AAD for 15 minutes at RT in the dark. Red cells were then lysed with Ammonium Chloride for 10 minutes. An equal volume of well-mixed FlowCount™ fluorospheres of known concentration were then added and then acquired on the flow cytometer without washing. PB CD34 results & collection CD34 results were calculated and reported as 10⁶/L and 10⁶/kg body weight respectively.

Linear regression analysis was performed to assess:

- overall power of the PB CD34 count (x10⁶/L) to predict the collection CD34 (x10⁶/kg)
- predictive power in patients mobilised using G-CSF alone compared to G-CSF/chemotherapy.
- predictive power in patients whose body weight was < 70kg compared to 70- 100kg and > 100kg
- predictive power in patients whose PB CD34 count was 0-20 x10⁶/L, compared to 21-50 x10⁶/L & > 50 x10⁶/L

RESULTS:

A total of 519 collections were analysed from 179 patients (refer table 1 for summary). Both the PB and Collection CD34 results demonstrated a skewed (non-normal) distribution and were therefore transformed to log scale prior to further statistical analysis.

Table 1 – Summary Data

Characteristic	Category	No Collections	%
Diagnosis	MM	185	35.6%
	NHL	133	25.6%
	AML	42	8.1%
	DLBCL	24	4.6%
	FNHL	21	4.0%
	CML	19	3.7%
	HD	18	3.5%
	CLL	10	1.9%
Other	67	12.9%	
Mobilisation	G-CSF	148	28.5%
	G-CSF + chemotherapy	371	71.5%
Weight class	< 70kg	156	30.1%
	70 – 100kg	318	61.3%
	> 100kg	45	8.7%
Weight (kg)	Median	76.00	
	Range	43 – 149	
PB CD34 (x10⁶/L)	Median	23.00	
	Range	0.20 – 1980.70	
Collection CD34 (x10⁶/kg)	Median	1.75	
	Range	0.01 – 97.13	

Linear regression graphical analysis:

Figure 1: Relationship between collection CD34 and PB CD34 for all samples (after log transformation).

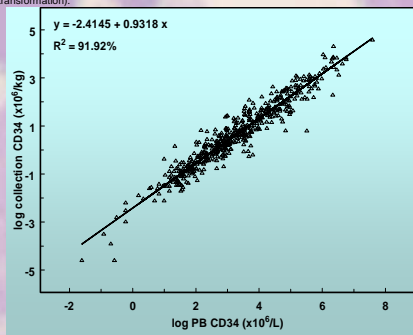


Figure 2: Relationship between collection CD34 and PB CD34 for samples from patients mobilised with G-CSF alone vs G-CSF+Chemo (after log transformation).

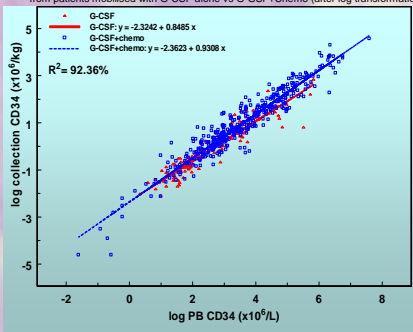


Figure 3: Relationship between collection CD34 and PB CD34 for samples from patients with weight <70kg vs 70-100kg vs >100kg (after log transformation).

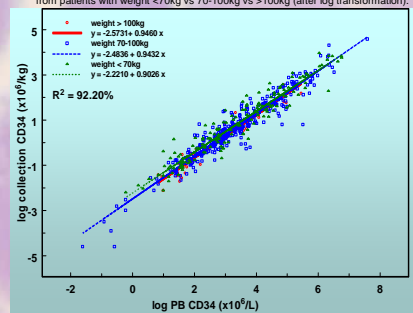


Figure 4: Relationship between collection CD34 and PB CD34 for all samples from patients with PB CD34 0-20x10⁶/L, n=242 (after log transformation).

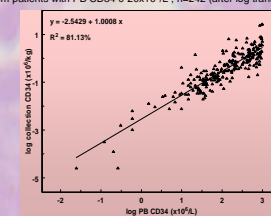


Figure 5: Relationship between collection CD34 and PB CD34 for all samples from patients with PB CD34 20-50x10⁶/L, n=113 (after log transformation).

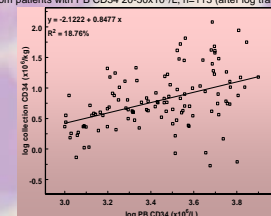
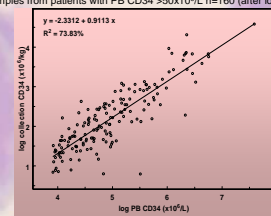


Figure 6: Relationship between collection CD34 and PB CD34 for all samples from patients with PB CD34 >50x10⁶/L, n=160 (after log transformation).



DISCUSSION:

Our results confirm previous studies which demonstrate very good predictive power of the PB CD34 for the collection CD34 result. Overall there is a significant linear relationship between collection CD34 and PB CD34.

The fit is significantly improved by adding into the model the effect of mobilisation. This indicates that collection CD34 levels are generally higher for G-CSF+chemo mobilisation and increase more quickly with an increase in PB CD34.

Analysis of varying body weights indicates that there is a difference in the mean levels of collection CD34 among the three weight classes, but the rate of increase of collection CD34 with PB CD34 does not differ significantly among the three weight classes (ie, similar slopes or parallel lines)

Analysis of varying PB CD34 ranges demonstrate an interesting finding - there is a significant linear relationship between collection CD34 and PB CD34 at low (0-20x10⁶/L) and high CD34 levels (>50x10⁶/L) however correlation is modest at moderate CD34 range (20-50x10⁶/L). This may be affected by the smaller sample size in this range.

CONCLUSION:

The use of PB CD34 analysis for prediction of resultant CD34 yield post PBSC collection remains an indispensable PBSC work-up assay. Caution must be demonstrated however, when reliance on PB CD34 counts to predict collection CD34 counts influences a decision on subsequent collection procedures prior to the availability of collection CD34 yield results. Our results confirm previous studies that show G-CSF/Chemo mobilization generally result in higher CD34 yields compared to G-CSF group alone. Additionally, we show that a specific PB CD34 count in the G-CSF/Chemo mobilisation group is likely to result in a higher CD34 yield compared to the G-CSF alone group. Ongoing monitoring of CD34 yield is important, unexpected outliers warrant further investigation.

REFERENCES:

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2. Keeney M, Chin-Yee I, Weir K, et al. Single platform flow cytometric absolute CD34+ cell counts based on the ISHAGE guidelines. *Cytometry* 1999;34:61-70