Associations Among White Blood Cells, CD34+ Cells And GM-CFU in Predicting The Optimal Timing of Peripheral Blood Stem Cell Collections by Apheresis

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Abstract
Aims: To analyze preapheresis blood CD34+ cells and corresponding apheresis products in order to investigate whether peripheral blood CD34+ cell counts correlate with peripheral blood progenitor cell (PBPC) yields and to determine the optimal timing for starting PBPC collections in our clinical setting.

Material and Methods: Thirty-eight patients with hematological malignancies and undergoing varied mobilization regimens were enrolled. White blood cell counts (WBC), CD34+ cells and granulocyte-macrophage colony-forming units (GM-CFU) enumeration were performed on blood samples taken immediately prior to each apheresis procedure and in the corresponding PBPC collection.

Results: A total of 61 apheresis procedures were performed, with a median of two collections per patient (range 1-3). The number of CD34+ cell/ml in the preapheresis blood correlated closely with CD34+ cells/kg and, to a lesser degree, with GM-CFU/kg in the apheresis products (r = 0.81 and r = 0.67, respectively, P <0.0001). WBC showed significant but poor correlation with CD34+ cells/kg and GM-CFU/kg (r = 0.43 and r = 0.45, respectively, P=0.004). A significant correlation was also found between CD34+ cells/kg and GM-CFU/kg in PBPC collections (r = 0.62, P <0.0001). Linear regression analysis indicated that the minimum threshold of 2 x 10^6 CD34+ cells/kg might be attained with a single apheresis if the CD34+ cells/ml in the peripheral blood measured prior to apheresis on the day of collection is ≥ 26 x 10^3 CD34+ cells/ml.

Conclusion: Collectively, these data demonstrate that circulating CD34+ cells is more useful than GM-CFU or WBC for predicting the optimal timing of PBPC harvests.
MATERIAL AND METHODS

Patients and PBPC mobilization

Thirty-eight patients (22 women and 16 men) undergoing varied mobilization regimens with rhG-CSF (Neupogen®, Amgen, USA) alone or with chemotherapy prior to PBPC transplantation were enrolled. The median age was 51 years (range 19-67 years) and the median weight was 70 kg (range 51-103 kg). Twenty-one patients had a diagnosis of non-Hodgkin’s lymphoma and 17 had a diagnosis of multiple myeloma. In all patients, leukapheresis was initiated when WBC was ≥ 2 x 10^6 /ml. Leukapheresis was performed using a COBE Spectra (Cobe Laboratories, Qedgeley, UK) apheresis system as described elsewhere. 4

Samples preparation

Immediately prior to each apheresis procedure, peripheral blood samples (preapheresis samples [PS]) were collected into EDTA anticoagulant. Samples from the leukapheresis products (leukapheresis samples [LS]) were collected into ACD anticoagulant. All samples were processed for WBC counts and CD34+ cells enumeration. GM-CFU were measured only in LS.

CD34+ cells enumeration

CD34+ cells were analyzed by flow cytometry as previously described. 4 Briefly, the cells were stained with PE-conjugated monoclonal antibody anti-CD34+ cells (HPCA-2, Becton Dickinson, Germany). Mouse anti-IgG1 was used as irrelevant isotype control. After red blood cell lysis and two washes, the cells were counterstained with propidium iodide (Becton Dickinson) before being analyzed in an ELITE flow cytometer (Coulter). A minimum of 100,000 events were recorded for each sample acquisition. Calculations were performed as follows: Total CD34+ cells = % CD34+ cells in LS x total WBC in LS x volume of leukapheresis product; CD34+ cells/kg = total CD34+ cells/patient’s actual weight; Preapheresis blood CD34+ cells/ml = % CD34+ cells in PS x total WBC in PS.

Clonogenic assays

GM-CFU were assayed in GEM1a methylcellulose semi-solid medium as already described. 4 Briefly, samples were adjusted to 2 x 10^6 nucleated cells/ml with HBSS calcium and magnesium-free (Gibco, UK). Aliquots containing 10^5 cells were seeded in triplicate on a 35 mm culture dish (Nunc, Denmark). Filtered supernatant from the human bladder carcinoma cell line 5637 was used as the source of growth factors. GM-CFU were scored after 14 days of incubation at 37°C in a humidified atmosphere with 5% CO2.

Statistical analysis

Relationships among study variables were assessed using Pearson’s correlation. Linear regression analysis was used to evaluate potential predictors of apheresis yield. A p-value less than 5% was considered statistically significant. All statistical analysis was performed using GraphPad Prism version 5.

RESULTS

All patients underwent apheresis when WBC was ≥ 2 x 10^6 /ml. The data were analyzed independently of patient’s diagnosis and mobilization schemes. A total of 61 leukapheresis procedures were performed, with a median of two collections per patient (range 1-3). A significant but weak correlation (r = 0.43, P < 0.004) was found between WBC in PS and CD34+ cells in LS. A weak correlation was also noted between WBC in PS and GM-CFU/kg in LS (r = 0.45, P < 0.004). In contrast, the number of CD34+ cells in PS was found to correlate strongly with CD34+ progenitor yields in LS (r = 0.81, P < 0.0001; Fig. 1) and to a lesser degree with GM-CFU/kg (r = 0.67, P < 0.0001). These results suggest that CD34+ cell levels in PS are a reliable predictor of harvest results, whereas WBC is less useful and an inferior predictor of expected CD34+ cells (or GM-CFU) in LS. A significant correlation (r = 0.62, P < 0.0001) was also observed between CD34+ cells/kg and GM-CFU/kg in LS, suggesting that both assays are valid measurements of PBPC yield in our clinical setting. Linear regression analysis indicated that the minimum threshold of 2 x 10^6 CD34+ cells/kg might be attained with a single leukapheresis if the CD34+ cells/ml in the PS on the day of collection is ≥ 26 x 10^6 /ml. Among study participants with preapheresis CD34+ ≥ 26 x 10^6 /ml blood, about 50% of the harvest products contained ≥ 2 x 10^5 CD34+ cells/kg.

DISCUSSION

Some investigators have reported a weak correlation between WBC in preapheresis blood and the total number of CD34+ cells collected, suggesting that a simple measurement of WBC is not an ideal surrogate for predicting progenitor cell yields. 2, 3 In addition, increased WBC counts in preapheresis samples do not necessarily correspond to peripheral expansion of

Fig. 1: Correlation analysis between preapheresis blood CD34+ cell counts and CD34+ cell content in apheresis product. The correlation coefficient and P-value are shown.
hematopoietic progenitor cells. Our data are consistent with findings previously reported in the literature and show a significant but weak correlation between WBC counts taken prior to leukapheresis and progenitor cell yields in the harvests. In agreement with recent studies, we also report a close correlation between CD34+ cells/ml in the preapheresis blood and CD34+ cells/kg in the harvests, suggesting that progenitor cell yields can be predicted from preapheresis blood samples by monitoring CD34+ cell levels during PBPC mobilization.6,7 We also observed a significant correlation between CD34+ cells/ml in the preapheresis blood samples and GM-CFU/kg in the harvest products, as well as a strong correlation between CD34+ cells and GM-CFU in leukapheresis samples. Unfortunately, the predictive role of clonogenic assays is of limited value because they remain poorly standardized between various laboratories and have the disadvantage of a 10- to 14-day delay before results are available. Therefore, colony assay measurement cannot be used to determine the timing of apheresis products. Analysis of CD34+ cell numbers in the peripheral blood and apheresis products showed that a minimum CD34+ level of 26 x 10^3 CD34+ cells/ml blood indicates that the threshold quantity of 2 x 10^6 CD34+ cells/kg is likely to be predicted by a single leukapheresis. In our study, about 50% of harvest products from subjects with ≥ 26 x 10^3 CD34+ cells/ml blood prior to apheresis contained at least 2 x 10^6 CD34+ cells/kg. The majority of leukapheresis products contained between 10-15 x 10^3 CD34+ cells/ml blood. Interestingly, the value of 26 x 10^3 CD34+ cells/ml blood is comparable to that reported by others despite the fact that CD34+ cells quantification by flow cytometry is not yet an inter-laboratory standardized test.6,7 However, it is important to note that in a proportion of patients a sufficient progenitor cell yield was not obtained even by repeated leukapheresis procedures.

In conclusion, preapheresis CD34+ cells quantification by flow cytometry on the day of harvest correlate significantly with progenitor cell yields and may be more useful than GM-CFU or WBC for predicting adequate PBPC collections.

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REFERENCES