Mobilization Characteristics and Strategies to Improve Hematopoietic Progenitor Cell Mobilization and Collection in Patients with Chronic Granulomatous Disease and Severe Combined Immunodeficiency

Abstract

G-CSF mobilized autologous hematopoietic progenitor cells (HPC) may be collected by apheresis of patients with CGD and SCID for use in gene therapy trials. An adequate HPC apheresis harvest requires robust CD34+ cell mobilization. We retrospectively evaluated CD34+ cell mobilization and collection in 73 consecutive CGD and SCID patients and in 99 age, weight and G-CSF dose-matched healthy allogeneic controls. In subjects aged $\leq$20 years, day 5 peak pre-apheresis circulating CD34+ counts were significantly lower in CGD and SCID than in control patients; mean peak CD34 = 59, 64, and 81 cells/uL, respectively, p=0.05. The SCIDs had lower CD34 collection efficiency than CGDs and controls; mean efficiency 40%, 63% and 58%, respectively, p=0.001. In subjects >20 years, the CGDs had significantly lower CD34+ cell mobilization than controls; mean peak CD34 = 41 and 113 cells/uL, respectively, p<0.0001. In a multivariate analysis, lower sedimentation rate (ESR) at mobilization was significantly correlated with better CD34+ cell mobilization, p=0.007. In SCIDs, CD34 collection efficiency was strongly correlated with higher red cell indices (MCV: R^2=0.77, p=0.004; MCH: R^2=0.94, p<0.0001; MCHC: R^2=0.7, p<0.007) but not hemoglobin. CGD and SCID populations are characterized by significantly less robust CD34+ HPC mobilization than healthy controls. The presence of active inflammation/infection as evidenced by ESR may negatively impact mobilization. Among SCIDs, markedly reduced CD34 collection efficiencies were related to iron deficiency. In these patients, decreased red cell size and density due to iron deficiency may impair apheresis cell separation mechanics.

Introduction

Primary immunodeficiency disorders (PINDs) such as Chronic Granulomatous Disease (CGD) and Severe Combined Immune Deficiency (SCID) are genetic disorders of the innate and adaptive immune systems, respectively. The resulting clinical syndromes may be life-threatening and in severe cases, patients present in childhood with recurrent infections, CGD-associated colitis, granulomas, and failure to thrive. Hematopoietic progenitor cell (HPC) transplantation from an HLA-matched sibling donor may be curative and is the treatment of choice in these conditions. Gene therapy may be a less toxic alternative if the genetic defect has been identified [1].

Since the discovery of their engraftment capability more than two decades ago, cytokine-stimulated peripheral blood progenitor cells (PBPCs) have surpassed bone marrow as the preferred source of stem cells for allogeneic and autologous transplantation [2, 3]. They have also been shown to be an efficient source of autologous cells for somatic gene therapy in CGD and SCID [4-6]. Compared to bone marrow aspiration, the relative ease of HPC collection by apheresis and larger CD34+ cell yields have made them the preferred cell source in gene therapy trials. Autologous PBPC collections may then be enriched by immune-affinity techniques and transduced with viral vectors carrying the missing genes to manufacture a clinically relevant dose of final product [1].

Factors influencing HPC mobilization and methods to maximize progenitor cell yield have been studied extensively in the HPC transplantation setting. Parameters shown to significantly affect mobilization in healthy allogeneic PBPC donors include donor age, sex, race, and total G-CSF dose administered during mobilization.[7]. Donor characteristics may also affect the collection efficiency of HPC apheresis procedures. In an analysis of 793 consecutive healthy allogeneic donors undergoing HPC apheresis procedures, red cell microcytosis was noted to impair cell yields independent of pre-apheresis CD34+ cell counts[8]. Lastly, in the setting of autologous

PBPC collection, mobilization failure (collection of less than 2 X 10^6 CD34+ cells/kg), commonly results from prior exposure to myelotoxic chemotherapy or radiation [9-12]. CD34+ cell mobilization responses may be improved in donors with limited marrow reserve by adding plerixafor to G-CSF [13, 14].

In patients with PINDs, chronic infections and/or inflammation can result in persistent leukocytosis. Inflammatory states have also been associated with increased endogenous G-CSF levels in mouse models [15]. Paradoxically, Sekhsaria et al. reported significantly impaired HPC mobilization in 18 CGD and 2 SCID patients compared to healthy controls [7]. They also noted the lack of an association between peak mobilized CD34+ cell counts and baseline absolute neutrophil counts among their subjects. The goal of the current study was to more fully characterize the CD34+ mobilization response to cytokine stimulation, in a larger cohort of CGD and SCID patients, and to identify and possibly mitigate the causes for poor CD34+ mobilization and collection yields in this population. We hoped in this manner to develop strategies to enable the collection of at least 5 x 10^6 CD34+ cells/kg, which is the typical targeted starting material for patients undergoing autologous HPC mobilization in gene therapy trials[16].

Methods

Cases and matched controls

We performed a retrospective case-control study with 73 consecutive CGD (n=63) and SCID (n=10) subjects who underwent PBPC collection from April 1997 to October 2012. The control population consisted of healthy sibling or unrelated donors participating in our allogeneic transplant protocols during the same period, and were matched by age, weight, and total G-CSF dose received. All subjects gave informed consent in accordance with the Declaration of Helsinki and the IRB–approved transplantation protocols. Baseline demographic data were collected on all participants. Subjects were divided into pediatric ($\leq$ 20 years) and adult (> 20 years) cohorts by age, based on prior observations that HPC mobilization responses differ in

children versus adults [26]. A higher than usual cut-off for the definition of pediatric age was used due to the growth retardation and low weight of many of the subjects. The pediatric group consisted of CGD (n=38) and SCID (n=9) cases and a 1:1 ratio of healthy controls (n=47). The adult group included CGD (n=25) and SCID (n=1) cases and a 2:1 ratio of controls (n=52), who were matched with cases by age, sex, weight, and total G-CSF dose. The availability of a larger number of matched controls for the older age group allowed a 2:1 comparison; this was not possible in the younger age group.

Ancillary medications in patients

Interferon-gamma (IFN-g) (1.5 mg/kg 3 times weekly) was withheld 6 weeks in advance in all except 7 CGD patients who continued to take it during HPC mobilization. For CGD-colitis management, oral prednisone was given to 12% of patients at therapeutic doses (5-10 mg daily) and to 42% at prophylactic doses (2.5-5 mg daily); 46% did not receive steroids during CD34+ mobilization. All patients received TMP/SMX and Itraconazole prophylaxis. Data were collected on the presence of active infection (bacterial and/or fungal) and/or CGD associated-colitis at the time of collection.

PBPC mobilization and collection

Patients and healthy controls received subcutaneous injections of G-CSF (filgrastim, Neupogen®; Amgen, Thousand Oaks, CA) for 5 consecutive days (10-16 mcg/kg/day). The actual dose administered was obtained from a review of pharmacy records. Circulating CD34+ cell counts were assessed by flow cytometry on day 4, on day 5 pre-apheresis, on day 5 post-apheresis. In a subset of patients (n=14) whose day 4 CD34+ count was below 20 cells/μL, a single dose of plerixafor (Mozobil, Genzyme, Cambridge, MA) 240 mcg/kg was given 10 hours prior to the start of apheresis.

PBPCs were collected by leukapheresis on day 5 of G-CSF. Three to thirty liters were processed per procedure based on the pre-apheresis CD34+ count as previously described [25]. Leukapheresis was performed with use of a CS-3000 Plus instrument (Fenwal Division, Baxter, Deerfield, IL). Prophylactic intravenous calcium and magnesium infusions were administered as previously described [17]. Collection efficiencies were calculated using the formula [25]:

$$\frac{\text{CD34+ content in product}}{\text{(mean of pre- and post- apheresis CD34+ counts) x (volume processed)}}$$

Laboratory data

Complete blood counts were measured at baseline before G-CSF administration (pre-G-CSF), and before and after PBPC collection (post-G-CSF, pre- and post-apheresis). In the patient group, data were collected on erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and fibrinogen within a week of collection, as indicators of active inflammation and infection. Serum ferritin and RBC indices were analyzed as indicators of iron deficiency. To determine whether RBC microcytosis was due to thalassemia trait or iron deficiency, an anemia index (Combined Cell Index – CCI) was calculated, as described previously[18].

Statistics

Significance tests comparing 2 groups were conducted with two-tailed, non-paired t tests. Multivariate analyses were performed using stepwise forward logistic regression, based on parameters of significance in univariate analysis, using a commercial statistics program (JMP, SAS Institute Inc.,Cary, NC). Proportions between 2 or more groups were compared using two-tailed Fisher exact test or chi-square analyses, respectively.

Results

Subject demographics and CD34+ cell yield

Demographic characteristics for subjects are shown in Table 1. The patients were predominantly Caucasian (CGD=78%; SCID=100%) and male (CGD=95%; SCID=100%). SCID were significantly younger (19 vs.13 years, p<0.05) and weighed less than the CGD patients (46 vs. 33 kg, p<0.04). When analyzed by age groups, the CGD, SCID and control subjects under 20 years were similar in mean age, BMI and total G-CSF dose administered (these parameters were matched *a priori*)(Table 2). There were significantly more non-Caucasians and females among the controls. Baseline laboratory data, were similar in the 3 groups, with the exception of Hemoglobin (lower in cases than controls) and ferritin levels (markedly lower among SCID than CGD subjects: 20 vs.134 ng/mL, p<0.02). The mean peak circulating CD34+ cell count just prior to apheresis was significantly lower among cases than controls (60, 64 and 81 cells/uL in CGD, SCID and controls, respectively, p=0.05). In SCID but not CGD subjects, CD34+ cell apheresis collection efficiencies were significantly lower than in healthy controls (63, 40 and 58% in CGD, SCID and control, respectively, p=0.001).

In the adult cohort (>20 years old), CGD and control subjects were similar in age, sex and total G-CSF dose received (matched *a priori*) (Table 3). The two subject groups differed by race and weight. Hemoglobin and MNC counts were significantly lower in CGD than control subjects. Mean peak circulating pre-apheresis CD34+ cell counts were significantly lower among the CGD than control subjects (41 vs.113 cells/uL, p<0.0001). CD34+ apheresis collection efficiency was similar in the two groups (67 vs. 59%, p=0.07).

CD34+ cell counts: G-CSF dose alone or with plerixafor

Among cases, a higher G-CSF dose was only associated with a trend toward better mobilization (mean peak CD34+ count at G-CSF 10 ug/kg = 40/uL and at G-CSF 16 ug/kg = 63/uL, p=0.07).

Within each age category, when stratified by G-CSF dose, cases mobilized more poorly than controls at both 10 ug/kg and 16ug/kg (Tables 4 and 5). As the pre-apheresis circulating CD34+ cell count is the strongest predictor of CD34+ cell yield, the CD34+ cell yield per liter processed was also significantly lower among cases than controls.

Mean day 4 circulating CD34+ cell counts in the patients who received G-CSF plus plerixafor were significantly lower than day 4 counts in patients receiving G-CSF alone (14 ± 6 vs. 41 ± 33 cells/uL, p<0.001). Day 5 counts increased 3 to 4-fold in patients who received supplemental plerixafor, such that day 5 counts were significantly higher in the G-CSF plus plerixafor group than in the G-CSF alone group (76 ± 36 vs. 49 ± 39 cells/uL, p<0.03)(Fig.1).

Factors affecting CD34+ cell mobilization

In univariate analysis of patients who received G-CSF alone, higher CD34+ cell counts on day 4 and day 5 of mobilization were strongly correlated with greater subject weight, higher baseline platelet count, lower ESR at mobilization, higher total dose of G-CSF received. Among controls, higher peak CD34+ counts were similarly associated with weight, platelet counts and G-CSF dose, but in addition, male sex and higher baseline MNC count were also significant contributors. In multivariate regression analysis, only a higher baseline platelet count and lower ESR at time of mobilization remained significantly correlated with CD34+ cell mobilization in the patients, p=0.01 and 0.007, respectively; total G-CSF no longer made a significant contribution. In contrast, in multivariate analysis of the control group, male sex, higher baseline platelets and MNCs and higher total G-CSF dose remained significantly correlated with mobilization efficacy. Mean ESR was significantly higher among subjects with active infection and/or CGD-associated colitis compared to those without these events (40 vs. 16 mm/hr, p=0.0001). Although 26% and 37% of patients had clinical evidence of active CGD-associated colitis, fungal/bacterial infections, no correlation could be established between these clinical diagnoses and peak CD34+ cell counts. Interferon use in the peri-collection period among the 7 CGD subjects,

steroid use and antibiotic administration correlated poorly with mobilization in the initial analysis and were excluded from the final multivariate model.

Factors affecting CD34+ cell collection efficiency

Multivariate analysis of all subjects showed no effect of CBC parameters and ESR, on CD34+ cell collection efficiency. Similarly device type, type of venous access, inlet flow rate, and volume processed were similarly not correlated with collection efficiency. When the analysis was limited to SCID subjects, however, univariate regression demonstrated that CD34+ cell collection efficiency was strongly correlated with higher red cell indices (MCV: R^2=0.77, p=0.004; MCH: R^2=0.94, p<0.0001; MCHC R^2=0.7, p<0.007; CCI: R^2=0.8; p<0.002) but not with Hemoglobin. This association was not observed in CGD subjects (MCV: R^2=0.005, p=0.7; RBC: R^2=0.0008, RDW: R^2=0.04, CCI: R^2=0.04, p=0.2, p=0.02) (Figures 2 and 3). As noted above, mean ferritin was 20 vs. 134 ug/L in CGD vs. SCID pts. Among controls with normal Hemoglobin levels, red cell indices were moderately correlated with collection efficiency similar to what was previously reported [8].

Multiple day collections

Thirty-four of 73 patients underwent repeat collections. Mean pre-apheresis CD34+ count on the second day of apheresis, after an additional dose of G-CSF on day 6, was 24% lower than on day 5 (34 vs. 45 CD34+ cells/uL, p=0.1). In 4 patients who underwent a third consecutive PBPC collection, mean pre-procedure CD34+ counts decreased to 18.5 cells/uL on the third day of apheresis.

Discussion

We demonstrate, in the largest population of such subjects studied to date, that patients with CGD and SCID undergoing HPC collection for gene therapy trials are characterized by significantly less robust CD34+ mobilization than healthy age- and weight-matched controls. In

the CGD population, a 27 to 37% lower peak CD34 response to conventional doses of filgrastim was seen in the <age 20 and >age 20 cohorts, respectively, compared with controls. In the SCID patients, a 21% decrease in peak CD34 response compared to controls was seen. Our control groups demonstrated a more vigorous peak CD34+ mobilization response, 81 cells/uL, than that previously reported in healthy donors [7], possibly because of the younger population of the allogeneic donors included in this case-control study. Our patient population consisted largely of children and young adults with lower than average body mass indices due to chronic illness; we attempted to eliminate the possible confounding effects of age, BMI, sex, and G-CSF dose by selecting matched controls. Our study also provides novel information on correlates of donor and procedural parameters that influence CD34 mobilization responses in patients with CGD and SCID. Higher baseline platelet count, perhaps reflecting more healthy marrow reserve [7, 19], was strongly correlated with better CD34 mobilization, whereas higher ESR was associated with poor mobilization responses. In contrast to the control group, male gender, increased weight and BMI, and higher total G-CSF dose were not associated with better CD34 mobilization.

The presence of active infection/inflammation at collection, as evidenced by an elevated ESR, appeared to be a unique contributor to decreased CD34+ cell mobilization among CGD and SCID subjects, despite similar levels of G-CSF-associated leukocytosis in both patient and control groups. The role of inflammatory markers such as TNF-a, IFN-g, IL-2, IL-6, and endogenous G-CSF in stem cell mobilization has been evaluated mainly in the setting of bone marrow failure syndromes such as aplastic anemia, and yielded mixed results[20, 21]. PINDs may represent an informative setting in which to study pathways involved in cytokine-mediated marrow suppression. From a clinical perspective, collection yields may be improved if patients undergo mobilization after maximum infection control, when possible. The value of using a higher G-CSF dose in the setting of persistent infection is questionable. It is likely that

endogenous increases in G-CSF levels under these conditions may result in tachyphylaxis and inadequate response to exogenous G-CSF [15]. IFN-g administration did not appear to influence CD34+ cell mobilization in our study, although it has previously been reported to impair mobilization [21, 22].

Preemptively adding plerixafor to G-CSF, based on circulating CD34+ count measurements obtained the day prior to apheresis, appears to be an effective approach to mitigating low CD34 mobilization responses in patients with active infections. Plerixafor, approved for use in HPC mobilization in patients with multiple myeloma and lymphoma, is associated with engraftment pace and durability similar to that seen following G-CSF mobilization alone, despite gene-expression profiling differences in cells mobilized with filgrastim vs. plerixafor alone[13, 23]. The effect of plerixafor on gene transduction efficiencies, engraftment and transgene expression has not been fully characterized. Initial investigations suggest that progenitor cells collected with plerixafor have unique transduction and gene expression characteristics, especially with use of newer lentiviral vector protocols [24].

Among SCID subjects, in addition to poor mobilization, we noted markedly reduced CD34+ cell collection efficiencies. We have previously noted that low MCV values due to iron deficiency can markedly impair the efficacy of MNC collection by apheresis even in subjects who mobilize well. Variability in red cell size and density, as evidenced by MCV and MCH values, can affect optimal optical interpretation and automated sampling of the white blood cell layer by the apheresis device. Our SCID subjects had significantly lower Hemoglobin and MCV levels than healthy controls, had profoundly low ferritin levels, and had a CCI characteristic of iron deficiency. They had iron deficiency anemia. In contrast, the mean ferritin level in the CGD group was elevated, suggesting that low Hemoglobin in this group was due to inflammation and the anemia of chronic disease, and the CGD group was characterized by normal CD34 collection efficiency during apheresis. It is likely that vigorous, timely oral or parenteral iron

replacement in SCID subjects with microcytosis due to iron deficiency would result in markedly improved apheresis collection efficiencies, and we have seen this in our practice.

Nearly 50% of patients with poor CD34 mobilization responses required more than one procedure to collect the targeted CD34 dose of at least 10 million CD34+ cells per kg, despite use of larger volumes processed (as many as 8 blood volumes per procedure). In these patients who underwent multiple consecutive daily collections, pre-apheresis CD34+ counts decreased with each collection, despite additional G-CSF doses. The majority of patients achieved targeted cell dose collections after the second procedure.

In summary, cytokine-assisted CD34+ cell mobilization from the marrow into the blood in patients with primary immunodeficiency disorders is significantly impaired compared with that in healthy allogeneic donors. Selective marrow suppression by inflammatory signals or possibly diminished marrow CD34+ cell reserve due to stem cell utilization for repair in these chronic conditions, may be responsible for the poor mobilization responses. Tachyphylaxis to exogenous cytokine stimulation due to increased endogenous G-CSF secretion may further impair mobilization response. A low baseline platelet count, high ESR, and microcytosis due to iron deficiency can be used to predict those subjects at greatest risk of poor mobilization. The two most effective strategies to mitigate poor mobilization and inefficient apheresis collection in this population include prospective correction of iron deficiency and careful monitoring of preapheresis CD34+ cell counts to determine which patients would benefit from supplemental plerixafor. Strategies used in other situations, such as a higher G-CSF dose, were ineffective in this population. Lastly, vigorous prospective control of infection/inflammatory states, which act as marrow stressors, may improve mobilization.

References

1. Aiuti, A. and M.G. Roncarolo, *Ten years of gene therapy for primary immune deficiencies.* Hematology Am Soc Hematol Educ Program, 2009: p. 682-9.
2. Neben, S., K. Marcus, and P. Mauch, *Mobilization of hematopoietic stem and progenitor cell subpopulations from the marrow to the blood of mice following cyclophosphamide and/or granulocyte colony-stimulating factor.* Blood, 1993. **81**(7): p. 1960-7.
3. Motabi, I.H. and J.F. DiPersio, *Advances in stem cell mobilization.* Blood Rev, 2012. **26**(6): p. 267-78.
4. Sekhsaria, S., et al., *Granulocyte colony-stimulating factor recruitment of CD34+ progenitors to peripheral blood: impaired mobilization in chronic granulomatous disease and adenosine deaminase--deficient severe combined immunodeficiency disease patients.* Blood, 1996. **88**(3): p. 1104-12.
5. Cassel, A., et al., *Retroviral-mediated gene transfer into CD34-enriched human peripheral blood stem cells.* Exp Hematol, 1993. **21**(4): p. 585-91.
6. Li, F., et al., *CD34+ peripheral blood progenitors as a target for genetic correction of the two flavocytochrome b558 defective forms of chronic granulomatous disease.* Blood, 1994. **84**(1): p. 53-8.
7. Vasu, S., et al., *Donor demographic and laboratory predictors of allogeneic peripheral blood stem cell mobilization in an ethnically diverse population.* Blood, 2008. **112**(5): p. 2092-100.
8. Wang, T.F., et al., *Poor harvest of peripheral blood stem cell in donors with microcytic red blood cells.* Transfusion, 2013. **53**(1): p. 91-5.
9. Chow, S., et al., *Predictors of unsuccessful mobilization with granulocyte colony-stimulating factor alone in patients undergoing autologous hematopoietic stem cell transplantation.* J Clin Apher, 2013.
10. Kuittinen, T., et al., *Prediction of mobilisation failure in patients with non-Hodgkin's lymphoma.* Bone Marrow Transplant, 2004. **33**(9): p. 907-12.
11. Popat, U., et al., *Impairment of filgrastim-induced stem cell mobilization after prior lenalidomide in patients with multiple myeloma.* Biol Blood Marrow Transplant, 2009. **15**(6): p. 718-23.
12. Tournilhac, O., et al., *Impact of frontline fludarabine and cyclophosphamide combined treatment on peripheral blood stem cell mobilization in B-cell chronic lymphocytic leukemia.* Blood, 2004. **103**(1): p. 363-5.
13. Jantunen, E. and G. Kvalheim, *Mobilization strategies in hard-to-mobilize patients with lymphoid malignancies.* Eur J Haematol, 2010. **85**(6): p. 463-71.
14. Jantunen, E. and R.M. Lemoli, *Preemptive use of plerixafor in difficult-to-mobilize patients: an emerging concept.* Transfusion, 2012. **52**(4): p. 906-14.
15. Quinton, L.J., et al., *The granulocyte colony-stimulating factor response after intrapulmonary and systemic bacterial challenges.* J Infect Dis, 2002. **185**(10): p. 1476-82.
16. Kang, E.M. and H.L. Malech, *Gene therapy for chronic granulomatous disease.* Methods Enzymol, 2012. **507**: p. 125-54.
17. Bolan, C.D., et al., *Controlled study of citrate effects and response to i.v. calcium administration during allogeneic peripheral blood progenitor cell donation.* Transfusion, 2002. **42**(7): p. 935-46.
18. Boulton, F., *Evidence-based criteria for the care and selection of blood donors, with some comments on the relationship to blood supply, and emphasis on the management of donation-induced iron depletion.* Transfus Med, 2008. **18**(1): p. 13-27.

19.   Ott, M.G., et al., *Mobilization and transduction of CD34(+) peripheral blood stem cells in patients with X-linked chronic granulomatous disease.* J Hematother Stem Cell Res, 2002. **11**(4): p. 683-94.

20.   King, K.Y. and M.A. Goodell, *Inflammatory modulation of HSCs: viewing the HSC as a foundation for the immune response.* Nat Rev Immunol, 2011. **11**(10): p. 685-92.

21.   Maciejewski, J., et al., *Fas antigen expression on CD34+ human marrow cells is induced by interferon gamma and tumor necrosis factor alpha and potentiates cytokine-mediated hematopoietic suppression in vitro.* Blood, 1995. **85**(11): p. 3183-90.

22.   Sato, T., et al., *Expression and modulation of cellular receptors for interferon-gamma, tumour necrosis factor, and Fas on human bone marrow CD34+ cells.* Br J Haematol, 1997. **97**(2): p. 356-65.

23.   Donahue, R.E., et al., *Plerixafor (AMD3100) and granulocyte colony-stimulating factor (G-CSF) mobilize different CD34+ cell populations based on global gene and microRNA expression signatures.* Blood, 2009. **114**(12): p. 2530-41.

24.   Uchida, N., et al., *Accelerated lymphocyte reconstitution and long-term recovery after transplantation of lentiviral-transduced rhesus CD34+ cells mobilized by G-CSF and plerixafor.* Exp Hematol, 2011. **39**(7): p. 795-805.

25.   Bolan CD,et al., *Prospective evaluation of cell kinetics, yields and donor experiences during a single large-volume apheresis versus two smaller volume consecutive day collections of allogeneic peripheral blood stem cells.* Br J Haematol. 2003 Mar;**120**(5):801-7.

26.   Fowler CJ, Yau YY, Bolan CD, Leitman SF. A*nalysis of donor demographic and laboratory parameters affecting CD34^{+} cell mobilization in healthy allogeneic pediatric peripheral blood stem cell (PBSC) donors.* Blood 2010;**116**(21): 2259A.

Table 1: Patient Demographics

Characteristic*	CGD	SCID	p-value
N	63	10	----
Male, n (%)	60 (95)	10 (100)	1.0
Age (years)	19 ± 10	13 ± 6	**0.02**
Race, Caucasian %	78	100	0.74
Weight (kg)	46 ± 20	33 ± 12	**0.01**
Height (M)	1.5 ± 0.3	1.4 ± 0.2	0.1
BMI	20 ± 5	18 ± 2	0.05
Peak CD34+ count per uL	52 ± 37	66 ± 55	0.8
CD34+ x 10^6 yield/liter processed	22 ± 15	17 ± 22	0.5

*Only first collections of first mobilization cycles were included in the data analysis. Descriptive data were provided for repeat collections and mobilizations in results. The single adult SCID patient >20 years was included in the overall analysis.

Table 2: Patient and Control Demographic and Laboratory Data (Age ≤20 years)

Characteristic*	CGD	SCID	Controls	p-value
N	38	9	47	----
Male, %	97	100	36	<0.0001
Age (years)	11.7 ± 5.4	11.8 ± 5.2	11.5 ± 5.0	1
Race, Caucasian %	73	100	42	0.02
Weight (kg)	34.7 ± 15.6	31.3 ± 11.2	36.1 ± 13.7	0.6
Height (M)	1.4 ± 0.3	1.3 ± 0.2	1.4 ± 0.2	0.6
BMI	17.5 ± 3.0	17.4 ± 2.3	17.7 ± 2.6	0.9
% Central Line	95	100	75	0.007
Volume processed (L)	11.5 ± 5.8	12.7 ± 5.7	12.6 ± 5.9	0.6
Baseline Hemoglobin (mg/dL)	11.8 ± 1.6	12.3 ± 1.5	11.5 ± 5.8	0.03
Baseline Platelets ($x10^9$/L)	300 ± 97	269 ± 134	273 ± 46	0.7
Baseline MNC count ($x10^9$/L)	2.4 ± 1.0	2.5 ± 1.4	2.6 ± 0.6	0.9
Total G-CSF dose (ug)	413 ± 192	412 ± 218	402 ± 187	1
Additional plerixafor, n	7	1	0	
Peak circulating CD34+ count/uL	59.6 ± 37	64.3 ± 57	81.3 ± 40	0.05
CD34+ $x10^6$ yield/liter processed	24.8 ± 15.3	16 ± 22.8	31.4 ± 16	0.02
CD34+ collection efficiency (%)	62.8 ± 12	39.8 ± 24	58.2 ± 12	0.001
Ferritin[$]	134	20	--	0.02

*Cases and controls matched by age and weight in a 1:1 ratio; [$]Ferritin data were unavailable in controls.

Table 3: Patient and Control Demographic and Laboratory Data (Age>20)

Characteristic[#]	CGD	Controls	p-value
N	25	52	-----
Male, %	92	94	0.7
Age (years)	29 ± 5.9	29(±6.8)	1
Race, Caucasian %	84	67	0.02
Weight (kg)	63 ± 12	70 ± 8	0.006
Height (M)	1.7 ± 0.1	1.7 ± 0.1	0.1
BMI	22 ± 4	24 ± 2	0.03
% Central Line	84	5	< 0.0001
Volume processed (L)	18.7 ± 4.7	18.2 ± 4.8	0.8
Baseline Hemoglobin (mg/dL)	12.8 ± 2	15.4 ± 1	0.0001
Baseline Platelets (x 10^9/L)	250 ±1 20	256 ± 46	0.9
Baseline MNC count (x 10^9/L)	1.7 ± 0.8	2.5 ± 0.7	0.001
Total G-CSF dose (ug)	761 ± 266	846 ± 191	0.1
Additional plerixafor, n	3	0	
Peak circulating CD34+ count/uL	41 ± 34	113 ± 73	<0.0001
CD34+ x 10^6 yield/liter processed	18 ± 14	45 ± 27	<0.0001
CD34+ efficiency (%)	67 ± 17	59 ± 15	0.07

1 SCID patient>20yrs not included in analysis; cases and controls matched by age, weight and G-CSF dose (ug/kg) in a 1:2 ratio.

Table 4: Effect of G-CSF dose on peak blood CD 34+ cell counts and CD 34+ apheresis yields in different patient cohorts (Age≤20)

Administered Drug & Dose*	CGD	SCID	Controls	p-value
G-CSF 10 mcg/kg				
N	17	5	37	
Weight (kg)	37±15	26 ± 8	35 ± 12	0.3
Total G-CSF dose	366 ± 151	267 ± 81	356 ± 131	0.3
Peak CD34+ count per uL	49 ± 26	51 ± 31	81 ± 44	0.01
CD34+x 10^6 yield/liter processed	22 ±13	11 ± 9	32 ± 17	0.01
G-CSF 16 mcg/kg				
N	12	3	10	
Weight (kg)	29 ± 15	37 ± 15	40 ± 18	0.3
Total G-CSF dose	467 ± 234	599 ± 243	574 ± 262	0.5
Peak CD34+ count per uL	64 ± 42	84 ± 102	81 ± 26	0.6
CD34+x 10^6 yield/liter processed	26 ±17	26 ± 41	31 ± 11	0.8

*Four patients who received a G-CSF dose of 5ug/kg were excluded from this analysis. 14 patients who received G-CSF plus plerixafor were analyzed separately.

Table 5: Effect of G-CSF dose on peak blood CD 34+ cell counts and CD 34+ apheresis yields in different patient cohorts (Age>20)

Administered drug & dose*	CGD	Controls	p-value
G-CSF 10mcg/kg			
N	13	31	
Weight	64 ± 13	71 ± 7	0.1
Total G-CSF dose	648 ± 127	730 ± 91	0.05
Peak CD34+ count per uL	24 ± 20	85 ± 53	0.0001*
CD34+x 10^6 yield/liter processed	13 ± 12	35 ± 18	<0.0001
G-CSF 16mcg/kg			
N	7	21	
Weight	69 ± 8	68 ± 11	0.9
Total G-CSF dose	1093 ± 121	1019 ± 169	0.2
Peak CD34+ count per uL	55 ± 48	155 ± 79	0.0009
CD34+ x 10^6 yield/liter processed	21 ± 17	59 ± 31	0.0005

*4 patients who received 5 ug/kg of G-CSF were excluded from analysis. 11 patients who received G-CSF plus plerixafor were analyzed separately.
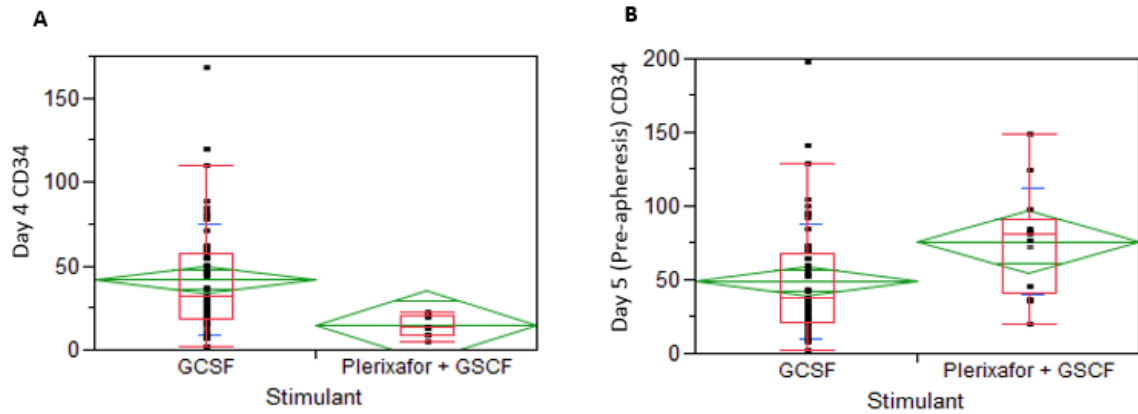
Figure 1: Effect of adding Plerixafor to G-CSF on CD34+ cell counts. (A) Day 4 CD34 counts were significantly lower in the group receiving plerixafor+ GCSF compared to those receiving G-CSF alone(p<0.05) (B) Day 5 (Pre-apheresis) count increased significantly in the plerixafor+ G-CSF group and was now also significantly higher than the group receiving G-CSF alone(p<0.05).
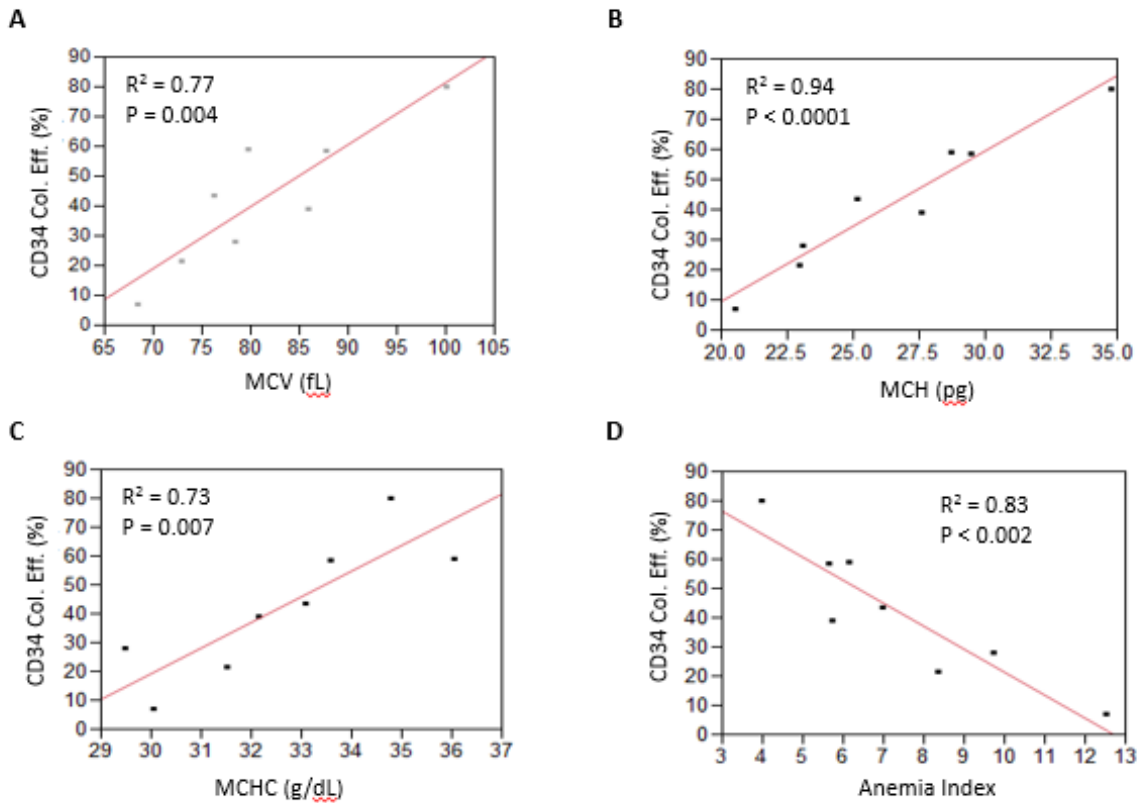
Figure 2: Effect of Iron Deficiency on Collection Efficiency in SCID Subjects. Panels A-D show red cell indices (x-axes) on the day of collection including Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and the Anemia Index/Combined Cell Index correlated significantly with CD34+ cell collection efficiency(y-axes) among SCID patients as described in the text. Anemia Index/CCI=RDWX10e4/(MCHXMCV); Higher the CCI, higher the chance of iron deficiency anemia.
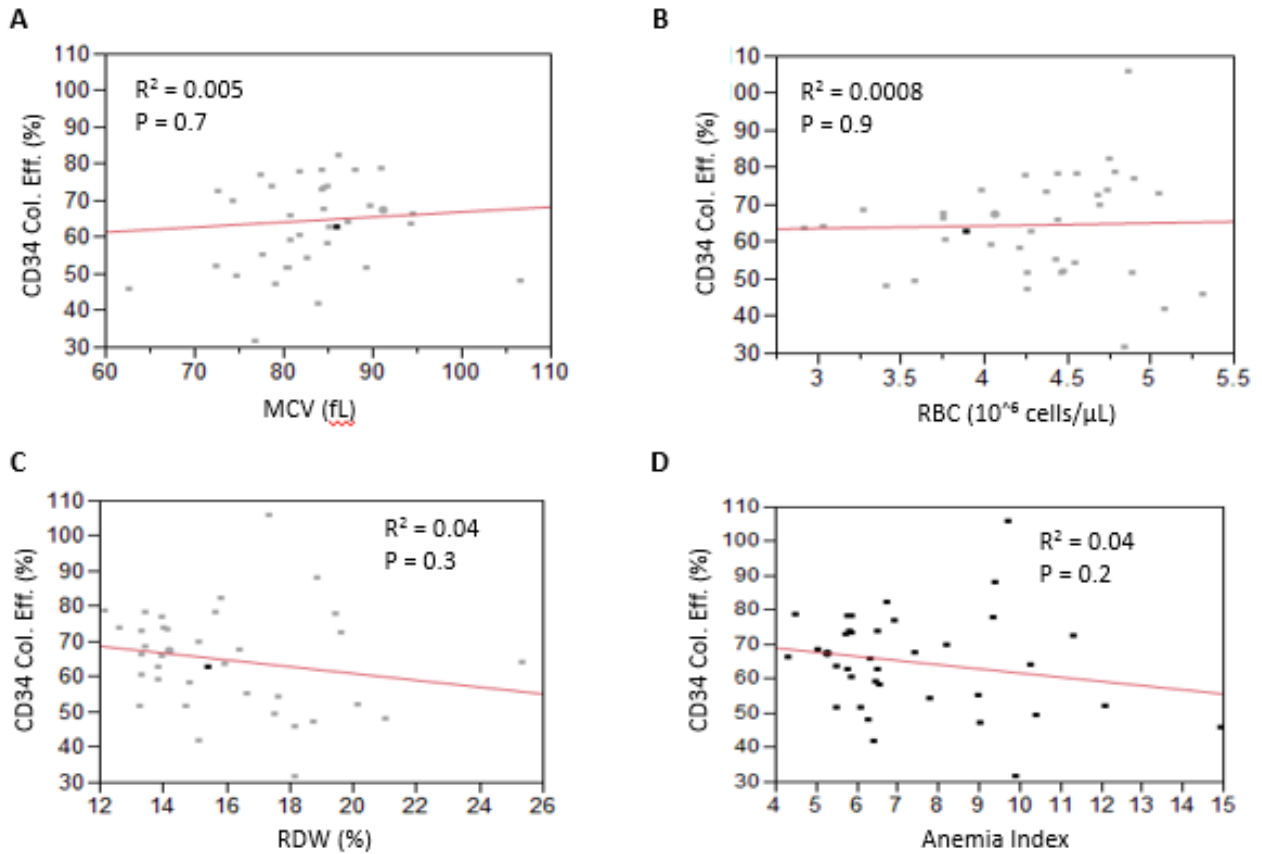
Figure 3: Effect of Iron Deficiency on Collection Efficiency in CGD Subjects. Panels A-D show the absence of any correlation between collection efficiency (y-axes) and red cell indices (x-axes), Mean Corpuscular Volume, RBC counts, Red cell Distribution Width and the Anemia Index/Combined Cell Index in CGD patients. The same was true for other indices including MCH and MCHC.