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MOBILIZATION AND COLLECTION OF PBSC IN HEALTHY DONORS: A RETROSPECTIVE ANALYSIS OF THE ITALIAN BONE MARROW TRANSPLANTATION GROUP (GITMO)

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ABSTRACT

Background and Objective. The number of allogeneic transplants of peripheral blood stem cells (PBSC) is rapidly increasing. Collection of PBSC in healthy subjects currently implies the administration of G-CSF or GM-CSF and, of course, the use of apheretic devices. These procedures involve potential risks, in particular the risk of leukemia secondary to growth-factor treatment. To evaluate the current practice of PBSC mobilization and collection, and initially assess the short-term side effects and efficiency of procedures, the GITMO (*Gruppo Italiano Trapianti di Midollo Osseo*) promoted a retrospective cooperative study among the Italian centers.

Methods. Seventy-six healthy individuals donating to their HLA-identical or partially matched sibling recipients in seven Italian centers form the basis of the present analysis. The data were retrospectively collected by proper forms, pooled and analyzed by means of a commercially available statistical soft package.

Results. All donors received G-CSF as mobilizing agent with different schedules according to each single center policy. A median of 2.5 (range 1-4) aphereses per donor were run. The most frequent side effect was bone pain. In no case did the medium term follow-up reveal subjective complaints or laboratory modifications. After G-CSF mobilization, WBC and lymphocytes counts increased to a maximum of (mean±SD) 48.1±15.6×10^9/L and 4.2±1.5×10^9/L, respectively. The peak was reached on day 5 in both cases. Platelets decreased after the apheretic procedures, reaching a minimum of (mean±SD) 77±26×10^9/L on day 8 and returning to normal values on day 11. Overall, the apheretic collection yielded (mean±SD) 18.6±19.2×10^8/kg donor body weight MNC; 10.4±5.7×10^6/kg $CD34^+$ cells; 90.6±75.9×10^4/kg CFU-GM and 4.3±1.8 ×10^8/kg $CD3^+$ cells. The target dose of 4×10^6/kg $CD34^+$ cells was harvested in 51.3% donors after a single apheresis, in 85.5% after the second, and in nearly 100% after a maximum of 3 aphereses.

Interpretation and Conclusions. These data demonstrate that collection of adequate numbers of circulating progenitors is feasible and well tolerated in healthy donors. However, only careful monitoring of donors and international cooperation will help to definitively assess the long-term safety of G-CSF for mobilization of PBSC.

Key words: PBSC, allogeneic transplantation, donor, G-CSF, mobilization, CD34

After initial pioneering attempts[1-3] and encouraging clinical results reported in the literature,[4,7,8] the number of allogeneic transplants of peripheral blood stem cells (PBSC) has increased rapidly. The EBMT registry listed only 12 allogeneic PBSCT in 1993, but their number increased to 180 in 1994, and to 537 in 1995 (Gratwohl, personal communication). The reason for this extraordinary success is probably an accelerated post-transplant recovery of blood counts, in particular of platelets.[4-6] The incidence and severity of acute GVHD seem comparable to that of mar-

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row transplants[4-9] but there is also hope of better disease control through an augmentation of the so-called GVL (graft-versus-leukemia) effect. This phenomenon has been documented in mice[10] but not in humans, and is supposed to be mediated by the large number of lymphocytes in the graft inoculum.

It is difficult at the present time to predict whether the use of PBSC will overcome that of bone marrow in allogeneic transplantation, similarly to what has already happened in the autologous setting. However, not only clinical results but also the safety of donors as well as the efficiency of cell mobilization and harvesting techniques remain critically important issues. Collection of PBSC in healthy subjects currently implies the administration of G-CSF or GM-CSF. These drugs are not completely devoid of side effects, nor is the use of apheretic devices. Of particular concern is a possible increased risk of leukemia development among donors after growth-factor treatment.

To evaluate the current practice of PBSC mobilization and collection, and initially assess the short-term side effects and efficiency of the procedures, the GITMO (*Italian Bone Marrow Transplant Group*) promoted a retrospective cooperative study among Italian centers. Data from 76 healthy donors, all receiving G-CSF for PBSC mobilization, where analyzed and are presented here. They point to the need for a large-scale, long-standing follow-up of donors for assessment of long-term risks, but show that collection of adequate numbers of progenitors is feasible and well tolerated.

Materials and Methods

Donor characteristics

Seventy-six healthy individuals donating to their HLA-identical or partially-matched sibling recipients in 7 Italian centers form the basis of the present analysis. The data were retrospectively collected by proper forms, pooled together and analyzed by two of us (I.M and A.M.C.) by means of a commercially available statistical soft package (SOLO 6.04). Thirty-four donors were male and 42 female and their median age was 37.5 years (range 6-67), with three donors being less than 18 years old (6, 7 and 14 years, respectively). Their median body weight was 65.5 kg (range 13-100). Twelve donors had previously undergone bone marrow donation under general anesthesia (Table 1).

PBSC mobilization and apheretic collection

For PBSC mobilization, all donors received G-CSF (filgrastim) but two, who received lenograstim. Dosage and schedules of G-CSF administration differed from center to center as well as individually within each single center. The majority of donors (n=49, 64.4%) received G-CSF 10 μg/kg/day for 5 to 7 days. A significant minority (n=13, 17.1%) received 15-16 μg/kg/day for 4 to 6 days, while 14 (18.4%) were assigned to an escalating-dose protocol, with 5 μg/kg/day for the first 3 days followed by 10 μg/kg/day for the following 3 days (Table 2). Day 0 was conventionally that of pre-treatment evaluation and day 1 the first day of G-CSF administration. Donors were monitored by different methods and at different times during G-CSF priming therapy, and criteria for starting apheretic collection were not reported by centers.

Table 1. Characteristics of the donors.

No. donors	76
Sex (M/F)	34/42
Age, median and (range)	37.5 (6-67)
< 18 years	3
Weight, median and range, kg	65.5 (13-100)
Previous BM donation	12

Table 2. Mobilization/collection methods.

		# of evaluable donors	%
Dose	10 mcg/kg x 5-7 d	49	64.4
	15-16 mcg/kg x 4-6 d	13	17.1
	escalating dose	14	18.4
Cell separator	continuous flow	51	79.6
	discontinuous flow	13	20.3
No. aphereses	1	7	9.3
	2	48	64
	3	19	25.3
	4	1	1.3

As reported in Table 2, apheretic collections were performed with an automated continuous-flow blood cell separator in 79.6% of the cases. Seven to 12 liters (median 9) of blood were processed at a time, with a median of 2.5 apheretic runs (range 1 to 4). Sixty-four percent of donors underwent 2 aphereses, 25.3% underwent 3, while only 9.3% were apheresed only once.

Results

Side effects

The whole procedure of G-CSF priming and apheretic harvest was generally well tolerated. There were no donor withdrawals. Sixty-six donors were evaluable for early side effects of G-CSF. As shown in Figure 1, the majority of them reported no or only mild complaints. Mild bone pain occurred in 59.6%, and occasionally required acetaminophen medication. A minority experienced cephalea and in 1% body temperature exceeded 38°C. There was no relationship between the G-CSF dose and the frequency or severity of symptoms. When asked, 23% of donors found the whole procedure to be very demanding but only 6% found it severely painful (Figure 2). Serum biochemistry modification consisted of a short-term elevation of ALT, LDH and alkaline phosphatase (data not shown).

We have follow-up data from 20 out of the 76 donors at a median of 16 (range 6-28) months from mobilization: all of them show normal blood counts and report no complaints.

Peripheral cell count variations

As shown in Figure 3, with the administration of G-CSF the WBC counts rapidly increased to a maximum of (mean±SD) $48.1\pm15.6\times10^9$/L on day 6. The maximum level of WBC exceeded 50×10^9/L in

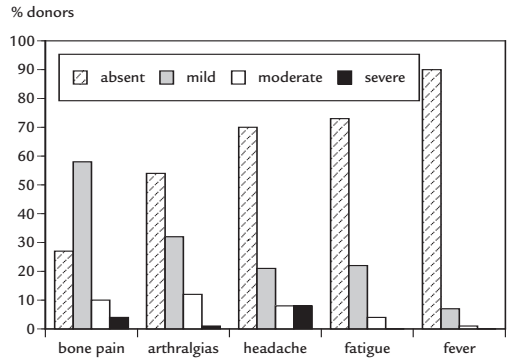

Figure 1. Occurrence of early side effects during G-CSF mobilization treatment, graded from absent to severe.

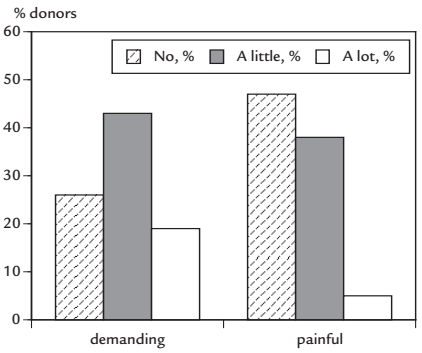

Figure 2. Donor (#=66) assessment of the entire procedure.

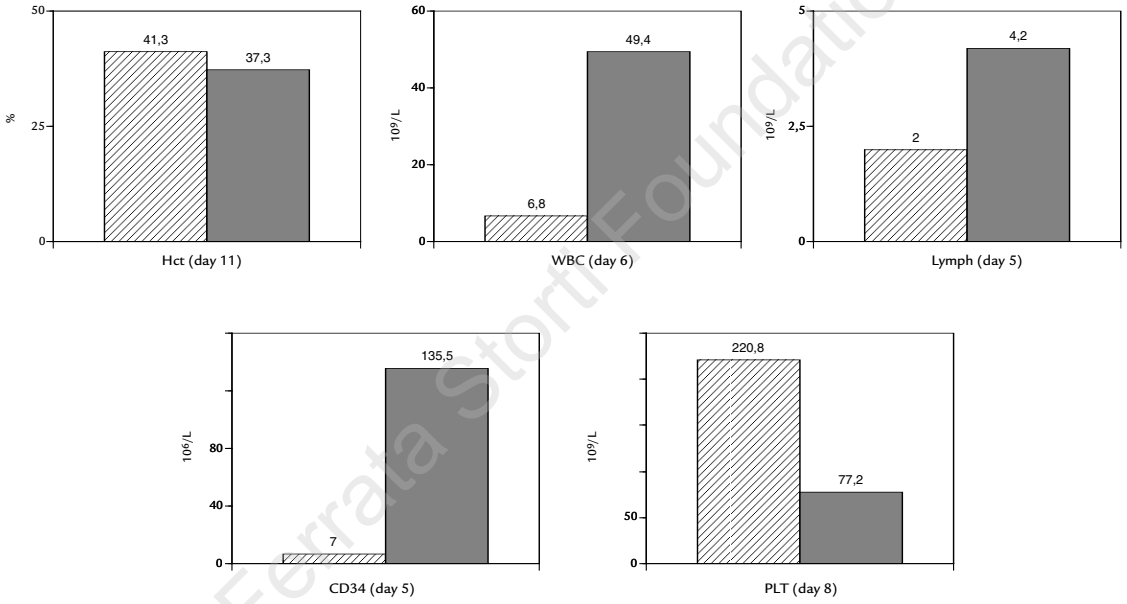

Figure 3. Cell count variations during and after G-CSF treatment. The shaded bars represent the base (pre-treatment) values, while the black bars represent the minimum or maximum level reached during the procedure. All values are expressed as means.

40% of donors and 70×10^9/L in 8%. The WBC counts returned to normal values on day 10. Not only the granulocyte, but also the lymphocyte counts increased, roughly doubling original levels, with a peak of $4.2\pm1.5\times10^9$/L on day 5. The monocyte counts did not change significantly. As expected, the platelet counts did not increase during G-CSF administration; however, the platelet counts showed a moderate fall following the apheretic procedures. The nadir was reached at day 8, with a count of (mean±SD) $77\pm26\times10^9$/L. As shown in in Table 3, the platelet counts fell below 70×10^9/L in 40% of the donors and below 50×10^9/L in 10%. The counts returned to $>100\times10^9$/L on day 11.

Progenitor cell mobilization

The kinetics of $CD34^+$ cell mobilization into the peripheral blood of healthy donors is shown in Figure 4. A peak rise in $CD34^+$ cells was noticed on day 5 following filgrastim treatment, with values of (mean±SD) $135.5\pm94\times10^6$/L. The increase in $CD34^+$ cells paralleled that of WBC, including lymphocytes. The level of $CD34^+$ cells rose 19 times above the base level, but rapidly declined following G-CSF discontinuation.

Cell harvest

The day of apheresis start was day 5 in 61% of the cases and day 4 in 28%, but there was a small

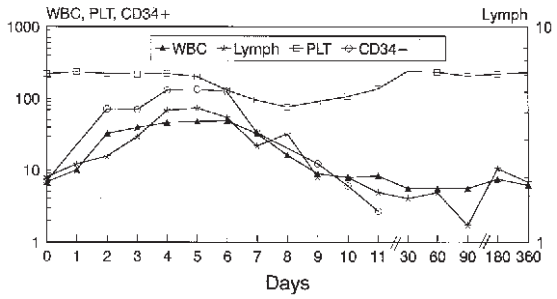

Figure 4. Curves of WBC, lymphocytes (Lymph), platelets (PLT) and CD34+ cells (CD34+) in the 76 donors. Values are expressed as means. Day 0 is that of pre-treatment evaluation, while day 1 is the first of G-CSF administration. Of the 76 donors, only 22 were evaluated on day 30, 7 on day 180 and 10 on day 360.

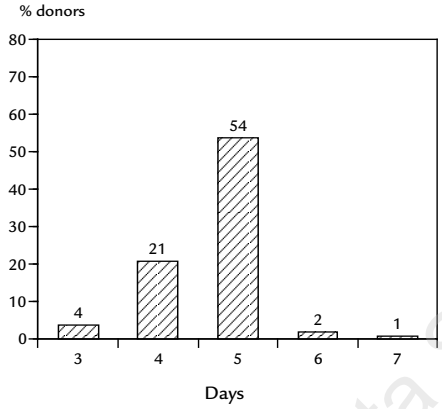

Figure 5. Day of apheresis start in the donor population.

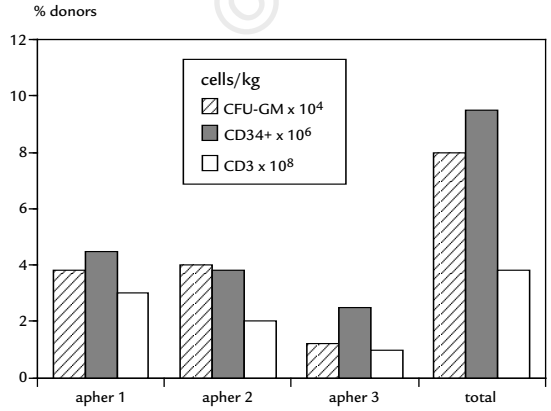

Figure 6. Yields of the apheretic collections.

Table 3. Occurrence of thrombocytopenia during and after apheretic procedures.

day	*6*	*7*	*8*	*9*	*10*	*11*
# evaluated donors	54	30	19	11	23	27
Plt < 70 x10⁹/L	5 (9.2%)	6 (20%)	8 (42%)	2 (18%)	4 (17%)	4 14.8%)
Plt < 50x10⁹/L	2 (3.7%)	2 (6.6%)	2 (10%)	0 –	1 (2.3%)	0 –

minority of donors who began their apheretic procedures earlier (day 3) or later (day 6 or 7) (Figure 5). Overall, the apheretic collections yielded (mean±SD) 18.6±19.2×10⁸ MNC/kg donor body weight, 10.4±5.7×10⁶/kg CD34⁺ cells, 90.6±75.9 ×10⁴/kg CFU-GM and 4.3±1.8× 10⁸/kg CD3⁺ cells (Figure 6). The CD34⁺ and the CD3⁺ cell yields decreased from the first to subsequent apheretic procedures, while the CFU-GM yield reached its maximum with the second apheretic run. There was no statistical difference in the CD34⁺ cell yield of the first apheretic procedure by day of apheresis start (day 4 vs day 5) or by G-CSF administration schedule.

Assuming a CD34⁺ cell dose of > 4×10⁶/kg as a reasonable progenitor number for allogeneic transplantation, 51.3% of donors reached that dose with a single apheresis, 85.5% after the second (Figure 7). Assuming a target of > 6×10⁶/kg CD34⁺ cells, the percentage of donors reaching that count with a single apheresis decreased to 37.5%. Interestingly, after the second apheresis no donor had a cumulative number of CD34⁺ collected < 2×10⁶/kg.

Discussion

Traditionally, the collection of progenitor cells for allogeneic transplantation involves multiple bone punctures at the iliac crests under general or lumbar anesthesia. This practice is accompanied by substantial discomfort for the donor, due in part to the need for hospital admission, to anesthesia or to harvest sequelae such as back pain and fatigue. Severe toxicity has been described in a small percentage of cases.[11,12]

The use of PBSC for allogeneic transplantation has been made possible by previous experiences in the field of autologous transplantation, where PBSC have rapidly replaced bone marrow, at least in some conditions.[13] For PBSC mobilization, the combination of chemotherapy and growth factors is most effective. In healthy donors the use of chemotherapy agents is not allowed for obvious ethical considerations. Collection of PBSC was initially attempted in steady phase,[1] i.e. without mobilization treatment, but this method is extremely impractical and uncomfortable for the donor since

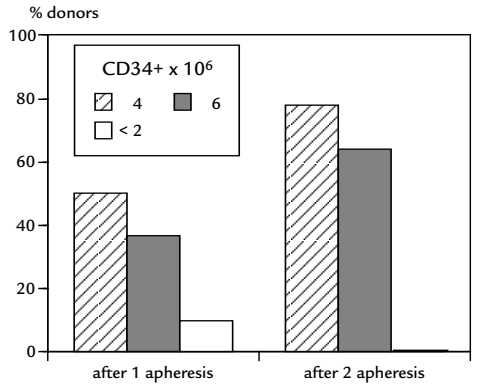

Figure 7. Probability of reaching given CD34+ cell counts in the apheretic product.

it requires a high number of apheretic procedures to reach a reasonable stem cell target. A number of studies demonstrate that G-CSF, when administered at a dose of 10 mg/kg/day or more, is able to mobilize a high number of progenitors into circulation[2,14-16] that are able to sustain long-term hematopoiesis. GM-CSF also retains this capacity,[17] but its mobilizing activity is less pronounced. Evidence of donor-derived hematopoiesis has also been obtained recently by the use of molecular probes over a year after PBSC allograft.[7,9]

In the present paper we analyze the data from 7 Italian centers concerning the mobilization and collection of PBSC from 76 healthy related donors. We show that the administration of G-CSF is well tolerated overall, with bone pain as the sole side effect reported by the majority of donors and seldom requiring medication. Laboratory enzyme modifications were transient; the elevation of alkaline phosphatase is due to increased neutrophil counts[18] not to liver toxicity. On the basis of our analysis, we confirm that in the short term PBSC collection after G-CSF priming is at least as safe a procedure as bone marrow harvest under general anesthesia.

We also show that, after initiation of G-CSF, WBC counts rapidly increase and reach their maximum on day 5. The WBC curve is almost superimposable on that of $CD34^+$ cells. These latter reach their peak on day 5, with a 12-fold increase over baseline values. We found no differences between different schedules and dosages of G-CSF, but this may be due to the large prevalence of donors receiving 10 to 16 µg/kg G-CSF versus those receiving lower doses.

When a standard mobilization regimen of G-CSF 10 to 16 µg/kg/day is employed, collections are best started on day 4 or 5 of G-CSF. A reliable parameter for actual collection yields is the absolute number of $CD34^+$ cells in the peripheral blood, as evaluated by flow cytometry.[19,20] In our analysis, on the basis of different methods of flow-cytometry evaluation according to individual center policy, $CD34^+$ cells $> 4\times10^6$/kg were obtained with two apheretic runs in over 85% of donors. A single run yielded that count in approximately half the donors. Since this is a retrospective study among multiple centers using different methods of mobilization and collection, these results can certainly be improved; however, it is worth considering that we did not observe true failures, since in no case did we have a yield $< 2\times10^6$/kg $CD34^+$ cells when at least two apheretic runs were performed.

At present we have no data concerning the late effects of G-CSF in healthy subjects. Though there is little or no fear that G-CSF or other cytokines may stimulate leukemia growth in normal subjects, there have been reports of leukemia development in neutropenic patients treated with G-CSF. The appearance of monosomy 7 followed by MDS/AML was observed in children with Kostmann's disease receiving high-dose filgrastim.[21] In this case, however, the underlying hemopoietic defect represents a pre-leukemic condition, and other neutropenias do not behave like that.[22] On the other hand, the risk of developing leukemia is higher among the relatives of affected individuals.[23] Hasenclaver and Sextro[24] reported that, in a *pessimistic* scenario, to demonstrate a 10-fold increase of leukemia more than 2000 donors would have to be followed for more than 10 years. Moreover, crude population-based incidence data would not to be sufficient as control, and a control group of bone marrow sibiling donors is probably necessary. This study can only be planned on an international basis. In conclusion, we show that G-CSF mobilization and PBSC collection are feasible and relatively well tollerated in healthy donors, but our study only represents the basis for the construction of a national donor registry with the aim of monitoring the efficency and side effects of currently used as well as forthcoming methods of PBSC mobilization.[25]

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