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PERIPHERAL BLOOD STEM CELLS FOR ALLOGENEIC TRANSPLANTATION. RECOMMENDATIONS FROM THE GITMO 1996

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**Gruppo Italiano Trapianti di Midollo Osseo, e di cellule staminali emopoietiche, nell'adulto e nel bambino. Commissione di Studio per l'impiego di cellule staminali periferiche da donatore sano e relativo trattamento con fattori di crescita*

ABSTRACT

Allogeneic transplants with PBSC are rapidly expanding, but a number of problems concerning both donors and recipients are still unsolved. GITMO (Italian Bone Marrow Transplant Group) has established a committee for allogeneic PBSC transplants. We present here an analysis of the main aspects of this evolving area and suggest revised guidelines for the use of allogeneic PBSC transplantation.

Key words: PBSC, transplantation, donor, apheresis, G-CSF

Allogeneic transplants of peripheral blood stem cells (PBSC) have met with extraordinary success during the last two years. The EBMT database registered only 12 such transplants in 1993, but their number jumped to 180 in 1994, and to 537 in 1995 (Gratwohl, *personal communication*). Following initial attempts, patients are now transplanted in an earlier disease phase, and allogeneic PBSC have also been used sporadically in the unrelated transplant setting.[1]

In 1995, in the 1st issue of this Journal the Committee presented guidelines for the clinical use of PBSC;[2] however, there are still a number of unanswered questions regarding both donors and recipients. Having examined the main aspects of this developing area, the Committee presents here an updated version of those guidelines.

Donor safety and compliance

A retrospective study recently conducted in several Italian centers[3] analyzed data from 55 donors receiving G-CSF for PBSC mobilization in view of an allogeneic graft. The side effects were minimal, with the exception of moderate bone pain reported by the majority of donors. A slight increase in ALT, LDH and ALP was recorded, but it was transient. Apheresis can lead to mild or moderate thrombocytopenia, which can be minimized by reinfusion of platelets recovered from the apheresis product. At a median follow-up of one year, donors have reported neither subjective problems nor presented laboratory modifications. However, only a minority of them were regularly monitored during the months that followed PBSC harvest. More data from the long-term follow-up of these donors are needed. The theoretical risk of leukemia after G-CSF treatment may be of concern to hematologists, but this finds little if any support from clinical practice. Leukemia is reported to occur *per se* more frequently among relatives of affected individuals.[4] This phenomenon has a genetical basis. An association of leukemia with given HLA C antigens, in particular Cw3 and Cw4,[5] has been described. This observation has no clearcut interpretation and might be the result of a selection bias, since the study included only patients entering complete remission. The development of monosomy 7

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followed by MDS/AML was observed with increased frequency in children with Kostmann's disease (infantile genetic agranulocytosis with eosinophilia) receiving high-dose G-CSF.[6] However, the same is not true for other neutropenias, suggesting that it is the underlying hemopoietic defect and not therapy with G-CSF that predisposes to MDS/AML.

In a recent conference on allogeneic PBSC held in Geneva, Hasenclever and Sextro[7] presented a preliminary study of long-term risks; in order to demonstrate a tenfold increase in leukemia risk, more than 2000 healthy PBSC donors would have to be followed for over 10 years. A control group of BMT donors of equal size would also be necessary. Such a study could only be carried out on a multi-national basis.

Whether children should be considered for G-CSF mobilization and PBSC donation is debatable. Though there may be a specific advantage in collecting PBSC from children in the case of considerable disparity with the recipient's body weight, we think that this practice should be discouraged in standard allogeneic transplants. This is also the opinion of the *Italian Association of Pediatric Hematology/Oncology* (AIEOP).

PBSC mobilization and collection

For PBSC mobilization the standard is currently represented by G-CSF, given subcutaneously at a dose of 10 μg/kg/day till the end of apheretic collections. This usually means 5 days of treatment. A higher dose may increase the yield of progenitor cells, but not to a significant extent.[8] A GITMO multicenter analysis[3] ascertained that $CD34^+$ cells peak on day 5 of G-CSF treatment. Although flow cytometry monitoring of the $CD34^+$ cell level is recommended, mobilization failure is extremely rare with an adequate dose of G-CSF and day 5 apheresis can be started even in the absence of actual data on the $CD34^+$ cell level.

GM-CSF has been employed alone or in association with G-CSF. At a dose of 10 μg/kg/day the side effects seem to be more pronounced than with G-CSF. The report of a more primitive population of progenitor cells being collected with GM-CSF[9] has not modified the use of G-CSF for PBSC mobilization in normal donors.

Apheretic procedures are run indifferently on continuous or discontinuous devices. These latter may be preferred when a single vein is available. The recommended standard is 1 or 2 collections on successive days. Since there is undoubtedly a certain discomfort related to multiple aphereses, an effort should be made to minimize their number. Because this also depends on the volume processed/run, it might be wise to increase it to 15 L (or 3 blood volumes) per run. On the other hand, though a *long* apheresis might be advantageous, the donor would be forced to endure a 5-hour procedure, which is probably just too much. The use of deep venous catheters is strongly discouraged and should only be envisaged as *extrema ratio* in donors whose venous access fails to perform adequately at the time of apheresis.

Cryopreservation of PBSC did not impair engraftment or increase GVHD in the study by Körblking *et al.*[11] The policy of cryopreserving PBSC offers the advantage of performing collection and transplantation at different times.

Graft-versus-host disease and T-cell depletion

In the clinical reports published so far,[10-14] the incidence and severity of acute GVHD with PBSC have been similar to those commonly observed with bone marrow. The incidence of chronic GVHD is more controversial, with an increased rate being reported in some studies;[13,15] however, GVHD is also a function of age, clinical status, prophylaxis protocol, infections and intensity of the conditioning regimen. Randomized studies are therefore recommended to give a definitive answer to the question of GVHD incidence, type and severity, but data from registries will also contribute through larger numbers of patients available for analysis.

Related to the problem of GVHD is that of T-cell depletion. In some reports of allogeneic PBSC transplants, T-cell depletion has been applied to the apheretic product to prevent acute GVHD. When depletion was performed by positive $CD34^+$ cell selection, there was no delay in the engraftment of granulocytes and platelets but there was an unexpected increase in acute

GVHD.[16] The degree of depletion, i.e. the reduction of the T-cell content of the graft sample, is of key importance for GVHD prophylaxis and abrogation of GVHD in mismatched family PBSC transplants has been demonstrated with $< 10^5$/kg $CD3^+$ cells. This number of $CD3^+$ cells is hardly obtainable from G-CSF primed apheretic products using a positive $CD34^+$ cell selection method alone. It is the opinion of this Committee that allogeneic PBSC transplants between HLA-identical siblings should be carried without depletion of T-cells and with standard (i.e. methotrexate-cyclosporine) GVHD prophylaxis.

Cell number and engraftment

The number of cells infused into the recipient is many times higher than with marrow. In a recently published study,[13] a median of 6.8×10^6/kg $CD34^+$, 2.5×10^8/kg $CD3^+$, 0.2×10^8/kg $CD56^+/3^-$ cells were infused into the recipient with 2 apheretic procedures. The conditioning regimen was BU-CY and GVHD prophylaxis consisted of MTX-CSA. Granulocytes $>0.5\times10^9$/L were reached in 13 days and platelets $>50\times10^9$/L in 15 days. A comparison with recovery times after marrow transplantation was performed only retrospectively[11] and demonstrated an advantage for platelets, but not for granulocytes.

The engraftment potential of PBSC seems to be superior to that of marrow. This is shown by their successful employment in the treatment of graft failure[17-19] and by the experience of the Perugia team with allogeneic mismatched transplants.[20] This latter study has clearly established that mobilized PBSC may be of value in overcoming the HLA barriers, reinforcing the concept that rejection is also counteracted by cell numbers. In a case of matched family pair transplant, engraftment was obtained after busulfan alone using G-CSF-primed PBSC.[21]

Following allogeneic PBSC transplantation engraftment is stable in the long term. This has been documented by chromosome and molecular studies[12,13] and represents the first demonstration in humans that mobilized peripheral blood contains true stem cells.

PBSC for unrelated or mismatched transplants

The number of unrelated volunteer donors in the registries has increased steadily during the last few years, with a parallel increase in the number of such transplants performed. The chance of obtaining stem cells for unrelated transplants without the need for general anesthesia is certainly appealing and would probably encourage many volunteers to subscribe. It would also be easier to expand in particular the number of donors belonging to ethnic minorities. Apheresis-derived mononuclear cells might be checked for $CD34^+$ cell and colony content, stored in liquid nitrogen and shipped when needed. The age limit for donors could also be expanded.[22] However, because of the limited experience with G-CSF mobilization in normal donors, National Marrow Donor registries have not approved the use of PBSC as first line. We expect this to be the case for the immediate future.

Recommendations

Donor

1. Related to the recipient, HLA identical or 1 antigen mismatched
2. Age limits 18-70 years, body weight >50 kg
3. No need for deep venous catheter(s)
4. Mobilization with G-CSF 10 μg/kg/day
5. Approved institutional protocol
6. Written informed consent
7. Long follow-up required

Recipient

1. Standard conditioning regimen and GVHD prophylaxis
2. Approved institutional protocol
3. Written informed consent

Procedure

1. Apheresis on day 5 (and 6) of G-CSF treatment
2. Reinfusion of autologous platelets when more than 2 aphereses are performed
3. Target of collections $>4\times10^6$/kg $CD34^+$ cells
4. No further manipulation in the case of transplants from HLA-identical siblings.

Conclusions

The results of allogeneic transplantation with PBSC are encouraging but there is still a long way to go. The main problems to solve are the safety of donors and the clinical benefit of this alternative stem cell source. While the first issue is difficult to answer, the second awaits the initial results of randomized trials in Europe and the USA that should be available in a year or so. Meanwhile, it is reasonable to continue considering allogeneic PBSC transplantation an experimental procedure to be pursued within approved study protocols. Concerning the use of PBSC for unrelated donor transplants, a common policy should be discussed and approved by international committees, with the participation of unrelated donor registries and donor associations. The Committee also formally invites those centers which are performing allogeneic transplants with PBSC in Italy to report their data regularly to the GITMO Registry in Genoa (Dr. A. Bacigalupo) and to cooperate with the Palermo BMT Center (Dr. I. Majolino), that is collecting data from healthy PBSC donors.

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