HIGH INCIDENCE OF CHRONIC GRAFT VERSUS HOST DISEASE AFTER ALLOGENEIC PERIPHERAL BLOOD PROGENITOR CELL TRANSPLANTATION

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Abstract

Background and Objective. The incidence of acute GVHD (aGVHD) in allogeneic peripheral blood progenitor cell transplantation (allo-PBPCT) seems to be similar to that seen in allogeneic bone marrow transplantation (allo-BMT). In contrast, some preliminary results suggest that the incidence of chronic GVHD (cGVHD) might be higher. The aim of the present study was to analyze the actuarial probability of developing cGVHD in allo-PBPCT, its clinical manifestations and response to treatment.

Methods. We have retrospectively analyzed clinical results from 21 allo-PBPCT recipients that had been transplanted at least 18 months before this study and that fulfilled the following criteria: HLA identical sibling donor, non T-cell depleted apheresis and more than 90 days of survival with sustained engraftment. The median follow-up was 12 months (range 4.5-22).

Results. Twelve out of the 21 (57%) patients presented cGVHD, 1 limited and 11 extensive. The actuarial probability of cGVHD was 72.7% (95% CI, 49-96%). The median interval from transplant to onset was 180 days (range 95-270). Nine of the

12 cases (75%) presented combined skin and liver involvement. Of the other three, the liver was involved in one case; skin, mouth, and nail cGVHD was observed in another case; and skin and mouth involvement together with an obstructive pulmonary disease was observed in the remaining case. Under therapy, a complete resolution of cGVHD manifestations was achieved in five cases, and a partial improvement was attained in three other cases. In two responsive patients, cGVHD reappeared after stopping treatment. Four patients were refractory to the treatment.

Interpretation and Conclusions. It would appear from this retrospective and multicenter study that, after a median follow-up of 12 months, cGVHD after allo-PBPCT could be more frequent than after allo-BMT. A randomized trial with a large number of patients and a sufficient follow-up will be necessary to answer this question definitively.
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The use of peripheral blood progenitor cells for allogeneic transplantation in humans is very recent: until 1993 only three cases had been reported.[1-3] It was soon evident that allo-PBPCT had two advantages over allo-BMT: its safe collection of hemopoietic progenitor cells, and the speed of neutrophil and platelet engraftment.[4,5] With a higher number of patients available, it seems that the incidence of severe aGVHD is not increased,[3-9] despite the large T lymphocyte content of PBPC grafts.[10,11] Two potential disadvantages, the possibility of unknown long-term effects of G-CSF and late bone marrow failure,[12] have not been confirmed.[7,13-15] However, one clinical issue of allo-PBPCT remains of concern: the possibility that the incidence of cGVHD is increased.[16,17] Until now, few allo-PBPCT

clinical studies have had a follow-up sufficient enough to address this question. To characterize cGVHD, we have retrospectively analyzed clinical results from 21 allo-PBPCT recipients that were transplanted at least 18 months before this study and that fulfilled the following criteria: HLA identical sibling donor, non T-cell depleted apheresis, and more than 90 days of survival with sustained engraftment. Analysis was focused on: a) actuarial probability of developing cGVHD, b) its clinical manifestations, and c) response to treatment.
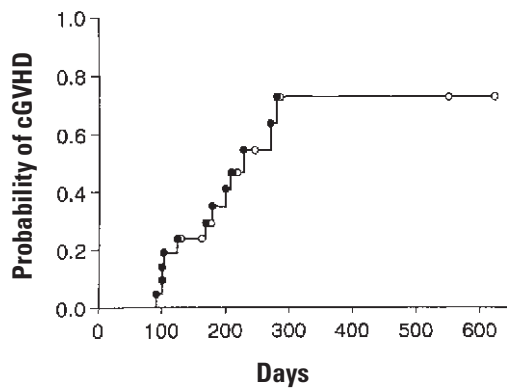
Patients and Methods

From the allo-PBPCT cases performed in Spanish centers between January 1994 and April 1995,[9] 21

Figure 1. Actuarial probability for chronic GVHD of the whole allo-PBPCT group. White circles, censored cases. Black circles, cases with chronic GVHD.

fulfilled the following criteria: HLA identical sibling donor, non T-cell depleted apheresis and more than 90 days of survival with sustained engraftment.

Donors

Twenty-one healthy donors (7 females, 14 males; median age: 35 years, range: 17-50) received G-CSF at a median dose of 10 µg/kg/day by subcutaneous injection for five to seven days. On day 5 after starting G-CSF, donors underwent 10-20 liter leukapheresis with a continuous cell separator.

Patients

The patients (9 females, 12 males; median age: 35.5 years, range 17-53) were diagnosed with AML (n=8), ALL (n=5), CML (n=2), CLL (n=2), NHL (n=1), HD (n=1), MM (n=1), and MDS (n=1). Six patients were at an early stage of their disease (ALL/AML CR1, CML CP1) at the moment of transplant, six at an intermediate stage (>CR2), and nine at an advanced phase (disease chemoresistant or in relapse, CML blast phase). Four cases (19%) were second transplants due to leukemic relapse. Growth factors were used post-transplantation in eight patients (38%). Cyclosporine A (CsA) and methylprednisolone (0.5 mg/kg days +7 to +14, 1 mg/kg days +15 to +28, and tapering the doses afterwards) (n=8), and CsA and methotrexate (MTX) (n=13) were used for GVHD prophylaxis. All patients were scheduled to continue CsA until day 180 after PBPCT. Patients received this preparative regimen: cyclophosphamide (Cy) (120 mg/kg) and total body irradiation (TBI) (12 Gy) (n=12), Cy (120 mg/kg) and busulfan (Bu) (16 mg/kg) (n=6), Bu (16 mg/kg) and melphalan (140 mg/kg) (n=1), TBI (12 Gy) and etoposide (60 mg/kg) (n=1), and BVAC regimen (n=1). The conditioning regimen was followed by the infusion of previously cryopre-

served (n=6) or non-cryopreserved (n=15) apheresis product as the sole source of stem cells. The sex parity donor to recipient was as follows: male to male (n=8), male to female (n=4), female to male (n=6), and female to female (n=3).

Diagnosis

The diagnosis and grading of acute and chronic GVHD were established according to the Seattle criteria.[18] Chronic GVHD was defined if GVHD was present after day 90.

Statistical methods

Students' t-test was used for comparison of groups with two-sided P-values. Actuarial curves for the whole group and for the different subgroups of patients were obtained by the Kaplan-Meier method and statistically compared using the Mantel-Cox test. Most statistical studies were performed employing the BMDP statistical software.[19]

Results

Donors and characteristics of PBPC collections

G-CSF administration was well tolerated except for moderate bone pain occurring in all donors. Leukapheresis procedures were performed without complications. Peripheral WBC median count immediately before harvest was 36×10^9/L (range, 26-62) and platelet median count after the last leukapheresis procedure was 110×10^9/L (range, 32-244). The median number of collected cells ($\times10^6$/kg) after a median of 2 (range, 1-5) leukapheresis per donor was: 5 (range, 2.4-13) CD34+ cells and 304 (range, 153-690) CD3+ cells.

Incidence of cGVHD

The median follow-up of the patients was 12 months (range, 4.5-25). At the time of the study, 13 patients had died, seven patients were alive in remission and one patient was alive in leukemic relapse. The median follow-up of the surviving patients was 20.2 months (18-25). Of the 21 patients, acute GVHD was clinical grade 0 (n=5), I (n=8), II (n=3), III (n=3) and IV (n=2). The actuarial probability for grade II-IV aGVHD was 38% (95% CI, 25-54%). Twelve out of 21 (57%) patients developed cGVHD (11 extensive and 1 limited). Actuarial probability for cGVHD of this group was 72.7% (95% CI, 49-96%) (Figure 1). There were two living patients that did not develop cGVHD after 551 and 623 days post-transplantation. The median day of the onset of cGVHD from transplant was 180 (91-280). It presented *de novo* in four (33%) and followed aGVHD in the other eight (quiescent in four and progressive in the other four). This complication was present in 4 out of 5 (80%) patients without previous aGVHD, in 4 of 11 (36.3%) with aGVHD clinical grades I-II, and in 4 out of 5 (80%) with grades III-IV. Chronic GVHD developed in three out of six

Table 1. Donor, graft and patient characteristics.

*Abbreviations: CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; CLL, chronic lymphocytic leukemia; NHL, non Hodgkin lymphoma; HD, Hodgkin's disease; MM, multiple myeloma RAEBt, refractory anemia with excess of blasts in transformation; Rel, relapse; GVHD, graft versus host disease; CR, complete remission; PR, partial remission; F, female; M, male; Values are expressed as 10⁶/kg for CD34⁺/CD3⁺ cells. NA, not available; PN, patient number; CsA, cyclosporine A; MTX, methotrexate; Pred, prednisolone; Cy, cytoxan; TBI, total body irradiation; Bu, busulphan; VP16, etoposide; Melph, melphalan; *days post-transplant.*

Table 2. Clinical characteristics of chronic GVHD after allo-PBPCT.

Abbreviations: CsA, cyclosporine A; Pred, prednisone; ATG, antilymphocyte globulin; *, days post-transplant;**, at the end of the study.

(50%) patients transplanted at an early stage of their disease, in three of six (50%) at an intermediate stage of disease, and in six of nine (66.6%) at an advanced phase. The type of GVHD prophylaxis did not influence the incidence of chronic GVHD. There was no correlation between the number of infused CD3^{+} or CD34^{+} cells and the development of cGVHD. Male recipients of PBPC from female donors had a higher probability of cGVHD (p=0.05).

Clinical manifestations of cGVHD

Nine out of the 12 cases presented combined skin and liver involvement, and most of them also presented mouth cGVHD (lichen planus or xerostomia). Of the remaining three cases, one presented skin, nail and mouth cGVHD, one patient showed skin, mouth and obstructive pulmonary disease, and finally, in one case, only the liver was involved. In those cases with liver involvement, a predominance of a cholestatic pattern was observed. Characteristics of the clinical manifestations of cGVHD of each patient are shown in Table 2.

Response to treatment

CsA and steroids (n=7) or steroids (n=5) were used initially for cGVHD treatment. The complete disappearance of all clinical manifestations of cGVHD was observed in five (41.6%) patients, although in two cases cGVHD reactivated after stopping treatment: one is in complete response again after prolonged low dosage of prednisone, and one developed extensive skin and facial involvement. A partial improvement was obtained in three cases, and they continued steroid treatment for 11, 9 and 7 months, respectively. Four patients had no response to the treatment. In three of these four non-responsive cases, cGVHD appeared as a progression of aGVHD and was quiescent in one. In two non-responsive patients, azathioprine (for three months) and ATG (10 mg/kg, ten doses), respectively, were added to the treatment, with no response. Three patients died directly due to cGVHD.

Discussion

In this series we analyzed the incidence of cGVHD in a group of 21 patients transplanted with allo-PBPC from HLA-identical sibling donors with non T-cell depleted apheresis and more than 90 days of survival. The median follow-up was 12 months. The results herein presented would suggest that the incidence of cGVHD after allo-PBPCT could be higher than that expected after allo-BMT. Twelve out of 21 (57%) patients in this series developed cGVHD, the actuarial probability being 72.7% (95% CI, 49-96%) (Figure 1). This incidence of cGVHD after allo-PBPCT is comparable to results from Anderlini *et al.*[16] and Majolino *et al.*,[17] and to other recent preliminary results.[20,21] A higher incidence of this complication in comparison with allo-BMT was not found in five other allo-PBPCT studies.[22-26] In two of them,[23,24] a historical group of patients transplanted with bone marrow was used for comparison, and no greater incidence of cGVHD was observed in allo-PBPCT. Of note, the median follow up of the allo-BMT group in one of these studies[24] was significantly longer (p = .001) than in the allo-PBPCT group; and in the other,[23] the median follow-up was not reported. In one study,[25] the incidence of cGVHD after a median follow-up of 6.5 months was 37%; remarkably, after a median follow-up of 12 months,[21] the incidence was 76%. This is similar to what occurred in our own experience, when after a short follow-up, only 36% of the patients developed cGVHD,[9] whereas after longer follow-up the figure rose to 72.7%. These results stress the importance of a long follow-up period in analyzing the incidence of cGVHD after allo-PBPCT.

In the IBMTR, the overall three-year actuarial probability of cGVHD among HLA-identical sibling allo-BMT was 45±2%.[27] Factors associated with a significantly increased risk of developing cGVHD were: the occurrence and severity of aGVHD, male recipients of bone marrow from alloimmunized female donors, and older patients.[26] In the present series, the actuarial probability for grade II-IV aGVHD was 38% (95% CI, 25-54%), the median recipient age was 35.5, and only in six out of 21 cases was the parity donor-to-recipient female to male. Thus, the high incidence of cGHVD in this series would not seem to be influenced by a high occurrence of previous aGVHD, increased patient age, or by a higher proportion of multipareous female donors.

There is now general agreement that the incidence of aGVHD is not higher in allo-PBPCT when compared with allo-BMT,[6-9] despite the large T lymphocyte content of PBPC grafts. It has been stated that, given a certain number of T-cells in the graft, the differences in minor HLA antigens rather than the amount of donor T lymphocytes would be a more important determinant for aGVHD.[28] Recent hypotheses suggest that the development of aGVHD after allo- BMT is the result of cytokine dysregulation: transplant conditioning regimen leads to the damage and activation of host tissues that secrete inflammatory cytokines.[29] This *cytokine storm* could offset, at least in part, the importance of the absolute number of donor T-cells. It has also been hypothesized that the activation of donor T-cells that secrete type-1 cytokines (Th1) preferentially induces aGVHD, whereas the activation of those that secrete type-2 cytokines (Th2) would lead to cGVHD.[30] Interestingly, murine studies have demonstrated that G-CSF administration may shift T lymphocytes from the Th1 to the Th2 cytokine pattern.[31] This could explain why the incidence of

aGVHD is similar (or even less) in allo-PBPCT compared to allo-BMT, whereas the incidence of cGVHD could be higher. Clinical studies on this issue are warranted.

Clinical characteristics of cGVHD in this series of allo-PBPCT did not differ substantially from those usually found after allo-BMT.[32] The skin, mouth and liver were the sites most frequently involved. One patient developed a skin and fascial syndrome and one case developed an obstructive pulmonary disease: these manifestations of cGVHD are also seen in 10% of allo-BMT patients.[32] The median interval from transplant to onset of cGVHD was 180 days (range 91-280), which was longer than the 111 days observed after allo-BMT,[33] and longer than that referred by others in allo-PBPCT.[16,17] Of note, in the IBMTR study,[33] late onset cGVHD was associated with a significantly higher probability of 2-year survival. This late occurrence of cGVHD in allo-PBPCT again stresses the importance of a long follow-up to clarify the actual incidence of this complication in allo-PBPCT. Although cGVHD was the primary cause of death in only three cases, it was a major cause of morbidity, and remained active in five cases. On the other hand, the scarce mortality associated with this disease improved the probability of long-term survival in a Seattle study,[34] and this effect became more evident in a group of patients with high probability of leukemic relapse.

In conclusion, although it is not possible to draw firm conclusions from this retrospective and multi-center allo-PBPCT study, it is suggested that a) incidence of cGVHD after allo-PBPCT could be higher than after allo-BMT, b) clinical characteristics of cGVHD in this series did not differ from those usually found after allo-BMT, and c) although cGVHD was the primary cause of death in only three patients, it was a major cause of morbidity. Until the actual frecuency of cGVHD in allo-PBPCT and its impact on survival and morbidity are known, T-cell depletion of the apheresis product[35-37] or prolonged CsA prophylaxis[38,39] may be considered for patients transplanted at the early phase of the disease.

References

1. Kessinger A, Smith DM, Strandjord SE, et al. Allogeneic transplantation of blood-derived, T-cell-depleted hemopoietic stem cells after myeloablative treatment in a patient with acute lymphoblastic leukemia. Bone Marrow Transpl 1989; 4:643-6.
2. Russell NH, Hunter A, Rogers S, Hanley J, Anderson D. Peripheral blood stem cells as an alternative to marrow for allogeneic transplantation [letter]. Lancet 1993; 341:1482.
3. Dreger P, Haferlach T, Eckstein V, et al. G-CSF-mobilized peripheral blood progenitor cells for allogeneic transplantation: safety, kinetics of mobilization, and composition of the graft. Br J Haematol 1994; 87:609-13.
4. Majolino I, Aversa F, Bacigalupo A, Bandini G, Arcese W, Reali G. Allogeneic transplants of rhG-CSF mobilized peripheral blood stem cells from normal donors. GITMO. Haematologica 1995; 80:40-3.
5. Majolino I, Aversa F, Bacigalupo A, Bandini G, Arcese W, Reali G. Peripheral blood stem cells for allogeneic transplantation. Recommendations from the GITMO 1996. Haematologica 1996; 81:529-

32.
6. Bensinger WI, Weaver CH, Appelbaum FR, et al. Transplantation of allogeneic peripheral blood stem cells mobilized by recombinant human granulocyte colony-stimulating factor. Blood 1995; 85:1655-8.
7. Schmitz N, Dreger P, Suttorp M, et al. Primary transplantation of allogeneic peripheral blood progenitor cells mobilized by filgrastim (granulocyte colony-stimulating factor). Blood 1995; 85:1666-72.
8. Korbling M, Huh YO, Durett A, et al. Allogeneic blood stem cell transplantation: peripheralization and yield of donor-derived primitive hematopoietic progenitor cells (CD34+ Thy-1dim) and lymphoid subsets, and possible predictors of engraftment and graft-versus-host-disease. Blood 1995; 86:2842-50.
9. Urbano-Ispizua A, Solano C, Brunet S, et al. Allogeneic peripheral blood progenitor cells transplantation: analysis of short-term engraftment and acute GVHD incidence in 33 cases. Bone Marrow Transpl 1996; 18:35-40.
10. Dreger P, Haferlach T, Eckstein V, et al. G-CSF-mobilized peripheral blood progenitor cells for allogeneic transplantation: safety, kinetics of mobilization, and composition of the graft. Br J Haematol 1994; 87:609-13.
11. Bensinger W, Price TH, Dale DC, et al. The effects of daily recombinant human granulocyte colony-stimulating factor administration on normal granulocyte donors undergoing leukapheresis. Blood 1993; 81:1883-8.
12. Goldman J. Peripheral blood stem cells for allografting. Blood 1995; 85:1413-5.
13. Anderlini P, Przepiorka D, Champlin R, Körbling M. Biologic and clinical effects of granulocyte colony-stimulating factor in normal individuals. Blood 1996; 88:2819-25.
14. Martínez C, Urbano-Ispizua A, Rozman C, et al. Effects of G-CSF administration and peripheral blood progenitor cells collections in 20 healthy donors. Ann Hematol 1996; 72:269-72.
15. Briones J, Urbano-Ispizua A, Rozman C, et al. Study of haemopoietic chimerism following allogeneic peripheral blood stem cell transplantation using PCR-STR. Ann Hematol 1996; 72:265-8.
16. Majolino I, Saglio G, Scimé R, et al. High incidence of chronic GVHD after primary allogeneic peripheral blood stem cell transplantation in patients with hematologic malignancies. Bone Marrow Transpl 1996; 17:555-60.
17. Anderlini P, Przepiorka D, Khouri I, et al. Chronic graft-vs-host disease (GVHD) after allogeneic marrow or blood stem cell transplantation [abstract]. Blood 1995; 86(Suppl 1):108a.
18. Thomas ED, Storb R, Clift RA, et al. Bone marrow transplantation. N Engl J Med 1975; 292:832-43.
19. Dixon WJ, Brown MB, Engelman L, Hill MA, Jenrich RT. BMDP statistical software. Berkeley: University California Press; 1988.
20. García-Conde J, Martín-Gaitero MJ, Arbona C, Benet I, Terol MJ, Solano C. High frecuency of chronic GVHD associated with unmodified allo-PBPCT. Preliminary results of allogeneic CD34+ selected cells PBPCT [abstract]. Blood 1996; 88(Suppl 1):261a.
21. Rosenfeld CS, Weinthl JW, Collins RW, et al. Does CD34 content of allogeneic peripheral blood cell transplants predict acute GVHD? [abstract]. Blood 1996; 88(suppl 1):616a.
22. Schmitz N, Bacigalupo A, Labopin M, et al. Transplantation of allogeneic peripheral blood progenitor cells from HLA-identical sibling donors. Br J Haematol 1996; 95:715-23.
23. Russell JA, Brown C, Bowen T, et al. Allogeneic blood cells transplants for haematological malignancy: preliminary comparison of outcomes with bone marrow transplantation. Bone Marrow Transpl 1996; 17:703-8.
24. Bensinger WI, Clift R, Martin P, et al. Allogeneic peripheral blood stem cell transplantation in patients with advanced hematologic malignancies: a retrospective comparison with marrow transplantation. Blood 1996; 88:2794-800.
25. Rosenfeld C, Collins R, Pineiro L, Agura E, Nemunaitis J. Allogeneic blood cell transplantation without posttransplant colony-stimulating factors in patients with hematopoietic neoplasm: a phase II study. J Clin Oncol 1996; 14:1314-9.
26. Miflin G, Russell NH, Hutchinson RM, et al. Allogeneic peripheral blood stem cell transplantation for haematological malignancies - an analysis of kinetics of engraftment and GVHD risk. Bone Marrow Transpl 1997; 19:9-13.
27. Bortin MM, for the international bone marrow transplant registry. Factors influencing the risk of acute and chronic graft-versus-host disease in humans: a preliminary report from the IBMTR. Proceedings of Leukemia 1988. Recent progress in biology and clinical research. First advanced international course, November 5-8, 1988. Bone Marrow Transpl 1989; 4(Suppl 1).
28. Dreger P, Glass B, Uharek L, Schmitz N. Allogeneic peripheral blood progenitor cells: current status and future directions. J Hematother 1996; 5:331-7.
29. Krenger W, Ferrara JLM. Dysregulation of cytokines during graft-versus-host disease. J Hematother 1996; 5:3-14.
30. Krenger W, Snyder KM, Byon JHC, et al. Polarized type 2 alloreactive CD4+ and CD8+ donor T cells fail to induce experimental acute graft-versus-host disease. J Immunol 1995; 155:585-93.
31. Pan L, Delmonte J, Jalonen CK, et al. Pretreatment of donor mice with granulocyte colony-stimulating factor polarizes donor T-lymphocytes toward type-2 cytokine production and reduces severity of experimental graft-versus-host disease. Blood 1995; 86:4422-9.
32. Sullivan KM. Graft versus host disease. In: Forman SJ, Blume KG,

Thomas ED, eds. Bone Marrow Transplantation. Boston: Blackwell Scientific Publications, 1994. p. 339-62.

33. Bortin MM, Rigden O, Horowitz MM, Rozman C, Weiner RS, Rimm AA. Temporal relationship between the major complications of bone marrow transplantation for leukemia. Bone Marrow Transpl 1989; 4:339-44.

34. Weiden PL, Sullivan KM, Flournoy NF, Storb R, Thomas ED. Anti-leukemic effect of chronic graft-versus-host disease. N Engl J Med 1981; 304:1529-33.

35. Urbano-Ispizua A, Rozman C, Martínez C, et al. Rapid engraftment without significant GVHD after allogeneic transplantation of CD34+ selected cells from peripheral blood. Blood 1997; 89:3967-77.

36. Bensinger WI, Buckner CD, Shanon-Dorcy K, et al. Transplantation of allogeneic CD34+ peripheral blood stem cells in patients with advanced hematologic malignancy. Blood 1996; 88:4132-8.

37. Finke J, Brugger W, Bertz H, et al. Allogeneic transplantation of positively selected peripheral blood CD34+ progenitor cells from matched related donors. Bone Marrow Transpl 1996; 18:1081-6.

38. Ruutu T, Volin L, Elonen E. Low incidence of severe acute and chronic graft-versus-host disease as a result of prolonged cyclosporine prophylaxis and early aggressive treatment with corticosteroids. Transplant Proc 1988; 20:491-3.

39. Bacigalupo A, Majolino A, Van Lint MT, et al. Cyclosporin A and chronic graft versus host disease. Bone Marrow Transpl 1990; 6:341-4.