

Donor cell leukemia (DCL): A prospective study of its identification and treatment

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Abstract

Donor-derived malignancies after allogeneic hematopoietic stem cell transplantation and after solid organ transplantation are considered as rare diseases. We have prospectively searched for donor cell leukemia in a 12-year period, in a single institution, in a group of 106 consecutive patients allografted because of leukemia. We have identified seven cases of donor cell leukemia; six were allografted because of relapsed acute lymphoblastic leukemia and one because of paroxysmal nocturnal hemoglobinuria/aplastic anemia. These figures suggest that the real incidence of donor cell leukemia has been underestimated. The six patients with lymphoblastic donor cell leukemia were treated prospectively with a pediatric-inspired combined chemotherapy schedule designed for de novo acute leukemia. A complete response was obtained in three out of six patients with lymphoblastic donor cell leukemia. It is possible to obtain favorable responses in donor cell leukemia patients employing combined chemotherapy. The long-term donor cell leukemia survivors remain as full chimeras and have not needed a second transplant. (Gac Med Mex. 2015;151:544-8)

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KEY WORDS: Hematopoietic stem cell transplantation. HSCT. Donor cell leukemia. DCL.

Introduction

In the cases of organ transplantations, malignancies originating in donor cells have been described after transplantations of both solid organs and hematopoietic stem cell transplantations (HSCT)¹⁻⁵. In HSCT, prevalences of donor cell neoplasms have ranged from anecdotic cases to figures as high as 5%¹ or more^{1,2}. Evolution in the capability to analyze donor-recipient chimerism by means of molecular analyses using

microsatellites (short-tandem repeats, STR) or flow cytometry has enabled detailed analysis of different donor and recipient hematopoietic cells populations after the HSCT, and for this reason, the detection of neoplasms originating in the donor has considerably increased^{1,2}. Leukemia relapses occurring in donor cells after allogeneic HSCT, also known as donor cell leukemia (DCL), have been previously reported in the literature¹⁻³ and some authors consider its incidence to be underestimated^{1,2}; lymphomas originating in donor cells have been less studied^{4,5}.

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Date of reception: 18-08-2014
Date of acceptance: 23-08-2014

In this study, we have prospectively identified the origin of post-transplantation leukemic activity in subjects undergoing allogeneic HSCT by using a reduced-intensity conditioning method developed in our country, known as “the Mexican method” for bone marrow (BM) transplantation. Additionally, patients prospectively identified as DCL carriers were evenly treated with combined chemotherapy (CT) as if they had de novo leukemias although, strictly, these are different malignancies to those that prompted the allogeneic HSCTs. The results of prospectively identifying DCLs and treating these neoplasms using a regimen for de novo leukemias motivated this report.

Material and methods

Patients

All consecutive patients transplanted with allogeneic hematopoietic stem cells at the Clínica Ruiz de Puebla between September 2002 and December 2013 were included. Written informed consent was obtained from both receivers and donors, and the procedures were authorized by the ethics and transplantations committees of the institution.

Transplants

In all cases, donors were identical-HLA (6/6 A, B and DR HLA antigens) or compatible-HLA (5/6 antigens) siblings. All patients were transplanted using the “Mexican method” with non-ablative conditioning⁶: oral busulfan, 4 mg/kg on days -6 and -5; i.v. cyclophosphamide 350 mg/m² on days -4, -3 and -2; i.v. fludarabine 30 mg/m² on days -4, -3 and -2. As graft versus host disease (GVHD) prophylaxis, oral cyclosporine A (CsA) 5 mg/kg was used from day -1 on, as well as i.v. methotrexate 5 mg/m² on days +1, +3, +5 and +11. CsA was maintained until day +180, with adjustments to obtain 150-275 ng/ml serum levels; subsequently, it was weaned in a 30-60-day period. If GVHD occurred, CsA was weaned more slowly⁶. All grafted patients were tested for chimerism using microsatellites or XY chromosomes enumeration in the cases with gender mismatch between donor and receiver.

Studies to define the origin of post-transplantation leukemic activity

All patients with leukemic activity after having been successfully transplanted or grafted were prospectively

studied to define the origin of the leukemia cells in BM, peripheral blood (PB) and cerebrospinal fluid (CSF). In the cases with less than 50% blasts, neoplastic cells were isolated by means of fluorescence-activated cell sorting (FACS) using flow cytometry and anti-CD45 and anti-CD10 antibodies labeled with fluorescein isothiocyanate and phycoerythrin, respectively². Cells weakly expressing CD45 and strongly expressing CD10 were selected using an electronic window with and EPICS Elite ESP flow cytometer (Coulter, Hialeah, FL). When a population of 100,000 selected cells was obtained, its purity was determined by re-testing using the same instrument and protocol. The selected cells had to have higher than 99% purity. The investigation of polymorphic markers (microsatellites) was conducted on the cell samples, isolated or not according to the number of blasts: cells were isolated with FACS if the number of blasts was lower than 50%².

Results

Patients and transplantations

Between September 2002 and December 2013, 106 patients with different types of leukemia were transplanted with allogeneic hematopoietic cells at the Clínica Ruiz de Puebla. The types of leukemia were 62 acute leukemias and 44 chronic leukemias; in the acute leukemia group, 29 were ALL in second remission. Of the transplanted ALL patients, 12 had leukemic activity after being successfully transplanted and grafted.

DCL

Seven DCL cases were prospectively identified. Six had been transplanted for ALL and one for paroxysmal nocturnal hemoglobinuria/medullary hypoplasia. Three of the seven patients have been previously reported^{2,3}. Age average of the patients who developed DCL was 16 years (3 to 36 years); five were females. Donors were in all cases identical-HLA (6/6 equal antigens, 6 cases) or HLA-compatible siblings (5/6 antigens, one case). The donors' median age was 16 years (ranging from 1 to 35). In three cases there was gender mismatch between receiver and donor. Table 1 describes patients and donors main characteristics.

Six of the seven cases developed acute B lymphoblastic DCL; one developed a B-type hairy cell leukemia variant³. The DCL appeared between 1 and 40 months after the allotransplant, with a median of 10 months. In the cases of those patients transplanted for ALL on

Table 1. Relevant characteristics of patients who developed leukemia in donor cells

| Case | 1 | 2* | 3* | 4† | 5 | 6 | 7 |
|---|--|-----------------------------|-----------------------------------|------------------------------------|--|---|--|
| Receiver | | | | | | | |
| Sex | Male | Female | Female | Female | Female | Female | Male |
| Age | 3 years | 6 years | 36 years | 34 years | 9 years | 16 years | 23 years |
| Diagnosis | B-ALL relapse | B-ALL relapse | B-ALL relapse | PNH/Medullary hypoplasia | B-ALL relapse | B-ALL relapse | B-ALL relapse |
| Donor | | | | | | | |
| Sex | Male | Female | Male | Male | Male | Female | Male |
| Age | 1 year | 16 years | 35 years | 27 years | 3 years | 12 years | 33 years |
| Compatibility | 5/6 | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 | 6/6 |
| aGVHD | No | No | No | No | Yes | No | No |
| cGVHD | No | No | - | Yes | No | Yes | No |
| DCL | | | | | | | |
| Time post-transplantation | 6 months | 16 months | 1 month | 40 months | 10 months | 32 months | 9 months |
| DCL | B-ALL | B-ALL | B-ALL | HCL | B-ALL | B-ALL | B-ALL |
| Leukemic cells % when DCL occurred | 65%, PB | 17%, PB | 35%, BM | 3%, PB | 100%, CSF | 100%, CSF | 10%, BM |
| Chimerism % when DCL occurred | 79% | 100% | 97% | 100% | 100% | 100% | 100% |
| Leukemia cells | | | | | | | |
| Receiver leukemia cells at diagnosis | CD 10, CD 19, CD 20, CD 45 | CD 10, CD 19, CD 20, HLA-DR | CD 10, CD 19, CD 45 | - | CD 2, CD 10, CD 19, CD 20, CD 34, CD 45, CD 79 | CD 10, CD 19, CD 20, CD 34, CD 45, HLA-DR | CD 10, CD 19, CD 20, CD 34, CD 45, CD 79 |
| Leukemia cells at DCL development | CD 10, CD 19, CD 20, CD 34, CD 38, CD 45 | CD 10, CD 19, CD 34 | CD 10, CD 19, CD 20, CD 34, CD 38 | CD 11, CD 19, CD 20, CD 25, CD 103 | CD 10, CD 19, CD 20, CD 34, CD 45, CD 58 | CD 10, CD 19, CD 20, CD 34, CD 45, CD 58 | CD 10, CD 19, CD 20, CD 34, CD 45, CD 58 |
| Evidence of leukemia cells donor-origin | MS | MS | MS, XY | MS, XY | MS, XY | MS | MS |
| Response | | | | | | | |
| Treatment | CT | CT | CT | (-) | CT + RT | CT * RT | |
| Remission | No | Yes | No | No | Yes | Yes | No |
| Current status | Dead | Alive | Dead | Alive | Alive | Alive | Dead |
| Time | 30 months | 98 months | 1 month | 64 months | 12 months | 11 months | 2 months |

ALL: acute lymphoblastic leukemia; PNH: paroxysmal nocturnal hemoglobinuria; aGVHD: acute graft-versus-host disease; cGVHD: chronic graft-versus-host disease; DCL: donor cell leukemia; CD: cluster designation; MS: microsatellites; XY: XX and XY cells enumeration; CT: chemotherapy; RT: radiotherapy; HCL: hairy cell leukemia.
 *published in reference number 2.
 †published in reference number 3.

second remission, the donor lymphoblastic leukemia cells showed similar expression of CD antigens, with minor modifications (see table 1). The number of blasts when the DCL became apparent was higher than 50% in three cases, whereas chimerism was higher than 90% in six of the seven cases; i.e., in all cases, more than 90% of white blood cells came from the donor of the transplanted hematopoietic cells. In cases 2, 3, 4

and 7, cells were separated with flow cytometry^{2,3}, while in the remaining 3 patients, the degree of leukemic infiltration in the presence of complete chimerism revealed the origin of the leukemic cells that became apparent post-transplantation in the donor. The analysis of the origin of the leukemia cells was carried out in BM in 3 cases, in PB in 2 cases and in CSF in 2 cases (See table 1). The demonstration of the origin of the

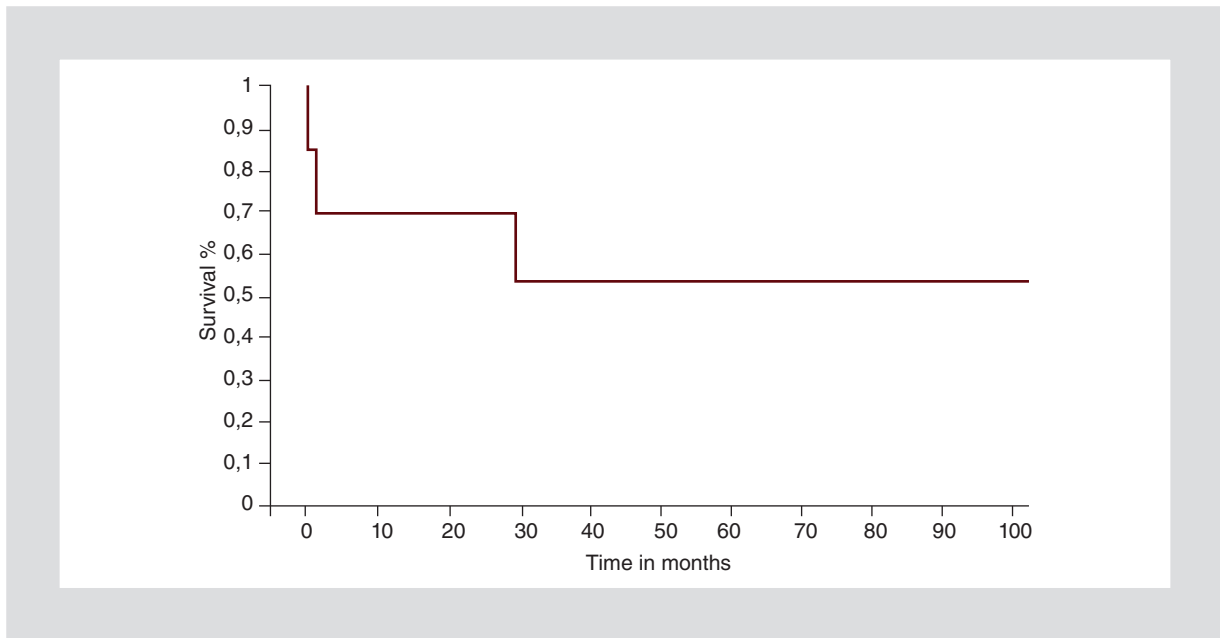


Figure 1. Overall survival of the seven prospectively-identified patients with donor cell leukemia according to Kaplan and Meier⁸.

leukemia cells appearing post-transplantation was made by microsatellites in all cases; additionally, in 3 cases it was made by chromosomes X and Y enumeration, when there was gender mismatch between donor and recipient (patients 3, 4 and 5).

Treatment of DCL

The female patient with donor cell-derived hairy cell leukemia (patient number 4) has remained stable without any treatment and was alive 64 months after the DCL diagnosis. The remaining 6 patients with donor cell-originated ALL were administered treatment with combined CT as if it was de novo ALL, with a regimen based on polychemotherapy whose details we have published previously, as well as the results of its use⁷. In all 6 ALL cases in donor cells, a modification of the TOTAL XI regimen was used⁷; in cases 5 and 6, who had leukemic leptomeningitis, brain radiotherapy was also used. ALL complete remission (CR) was achieved in 3 of the 6 patients; 2 patients died due to toxicity 1 and 2 months after CT was started and one patient died 30 months after the DCL diagnosis as a result of leukemic activity without achieving DCL CR. Three of the 6 ALL treated patients are alive on CR 11, 12 and 98 months after the DCL treatment start. In total, 4 of the 7 DCL patients are alive, with overall survival according to Kaplan-Meier being 54% at 98 months (Fig. 1).

Discussion

Neoplasms originating in donor cells after allogeneic HSCT are unique models for clonal evolution analysis of human tumors *in vivo*. Some authors have suggested that the appearance of donor cells-derived neoplasms probably occurs more frequently than originally was considered^{1,2}, whereas others do not support this theory⁹. Demonstration of the origin in donor cells can be complicated, with this being one of the probable causes for infrequent identification. The investigation of the origin of the leukemia cells appearing post-transplantation in the presence of chimerism is not routinely performed in institutions involved with the practice of HSCT, which is why we have considered its incidence to be underestimated¹⁻³. There are no prospective studies on DCL identification, with this series of patients being the first to prospectively and intentionally seek the origin of leukemia cells appearing post-transplantation and which nearly all physicians interpret as a relapse of the same leukemia that prompted the transplantation without further investigating the origin of the malignant cells. Hertenstein et al.¹⁰ describe 14 DCL cases in a total of 10,489 allogeneic HSCT procedures carried out in Europe, a figure equivalent to an estimated incidence of 124 DCL cases for each 100,000 transplantations. However, this study has its limitations, with the most relevant being that the

authors asked several BM transplantation centers for identified DCL cases and, therefore, the study did not prospectively analyze the occurrence of this complication. On the other hand, in a group of 40 patients with acute leukemia transplanted in a single institution and prospectively analyzing the characteristics of the leukemia cells of all patients at relapse, we identified two cases with this complication², which indicates a higher estimated incidence of DCL per 100,000 transplantations when compared with previously suggested figures¹⁰. These differences can be explained because, in the European study, transplantation centers were recruited to offer information on cases they had identified¹⁰, whereas in the other we did prospectively look into the development of this complication². Another difference is that, in the European multi-center study, patients were transplanted using myeloablative conditioning methods⁹, while in our single-center study patients were transplanted using a reduced-intensity conditioning regimen². Ever since we started to routinely investigate the possibility of DCL in all patients with leukemic activity after allogeneic transplantation, we have found 7 DCL cases in a total of 106 transplantations. Interestingly, in 62 cases of transplantation for acute leukemia, we identified 6 DCL cases, and in the group of 29 transplanted patients with ALL, 12 had leukemic activity after successfully being transplanted and grafted, out of which 6 were DCL and 6 were true relapses of the receiver's leukemia. The data here presented support observations previously made by us on underestimation of the real incidence of DCL¹⁻³, in a smaller group of until then transplanted patients.

From the therapeutic point of view, there are no studies prospectively analyzing the treatment of DCL; some DCL patients have been successfully treated using combination CT, while others have received a second transplantation^{1,2,9,10}. Generally, the use of CT for the treatment of DCL can induce remissions, but treatment-associated mortality is high; a second HSCT can induce prolonged remissions in some patients². Since we consider DCLs to be strictly de novo leukemias, we decided to prospectively use a treatment similar to that for de novo leukemias in all patients with lymphoblastic DCL, a regimen we used in 6 of the 7 reported cases, since the other case is a hairy cell leukemia that has not required treatment³. With this type of treatment, we obtained a CR in 50% of the patients, who are alive, free of leukemia and 100% chimerized 11, 12 and 98 months after having started the treatment of the DCLs. Since chimerism in these patients was always complete (higher than 90%), we considered there was no reason to transplant them again.

Interestingly, as many previous studies have indicated, donors whose cells became leukemic in the receiver have not developed neoplasms and all remain alive and in good health conditions.

The reasons why normal donor hematopoietic cells become leukemic in the receiver are not fully understood, but several possible mechanisms have been suggested: Sustained antigenic stimulus of lymphoid cells, aberrant homeostasis inducing or promoting leukemic transformation, deficient immune surveillance, hybridization of normal transplanted cells with malignant residual cells of the receiver, infectious agents, mutagenesis caused by CT, replicative stress, first hit in the donor followed by second hit in the receiver, etc.¹. These mechanisms are more extensively discussed somewhere else¹.

To summarize, in a single institution and in a 12-year period, we have carried out a study to prospectively identify patients who develop DCL after allogeneic HSCT; our findings suggest that the real incidence of this complication has been underestimated. Additionally, and also prospectively, we have treated the patients with lymphoblastic-lineage DCL with the same regimen designed for patients with de novo leukemia, taking into account that these are different neoplasms than those that motivated the allogeneic HSCTs. The results of this treatment are promising and suggest that up to 50% of patients with DCL can have favorable responses and leukemia-free prolonged survivals.

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