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Case report

Leukemia in donor cells after allogeneic hematopoietic stem cell transplant

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Summary:

The development of leukemia in donor cells after allogeneic hematopoietic stem cell transplant is an extremely rare event. We report here the case of a patient who developed myelodysplastic syndrome/acute myeloid leukemia, in cells of donor origin 3.5 years after related donor HSCT for refractory chronic lymphocytic leukemia and therapy-induced myelodysplastic syndrome. The origin of the leukemia was determined by analysis of minisatillite polymorphism tested on CD34$^+$ cells.

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Therapy-induced myelodysplastic syndrome (t-MDS) and acute myeloid leukemia (t-AML) are two well described complications affecting between 8 and 12% of patients who receive cancer treatment with alkylating agents and/or radiation therapy.[1,2] Clonal chromosomal abnormalities including numerical and structural chromosomal rearrangements are identified in the majority of these t-MDS/t-AML patients. Among those patients treated with radiation therapy and alkylating agents, loss of 5q and 7q predominate, whereas among those patients treated with topoisomerase II inhibitors, 11q23 and 21q22 abnormalities predominate.[3,4]

Allogeneic hematopoietic stem cell transplant (HSCT) is the only potentially curative therapy for MDS/AML.[5] The prognosis and the outcome after HSCT of t-AML is poorer than the *de novo* presentation;[6] in MDS, the prognosis seems to be influenced by the morphologic subclassification.[7] However, the outcome of t-MDS after HSCT seems to be similar to *de novo* MDS.[8]

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The development of leukemia in donor cells after allogeneic HSCT is a rare event that was first reported in 1971.[9] Cytogenetic analysis provided the basis for the diagnosis of the first few cases until molecular engraftment studies became available in the mid 1980s.[10] Currently, polymerase chain reaction amplification of minisatellite markers provides more accurate and faster determination of donor engraftment.[11] The pathogenesis of the development of leukemia in donor cells is not yet clear, but donor–recipient cell fusion, transfer of genetic material from recipient to donor cells, and marrow microenvironment abnormalities have been suggested as potential mechanisms.[12]

We report here the case of a 45-year-old male who developed donor marrow MDS/AML 3.5 years after related donor HSCT for refractory CLL and therapy-induced MDS.

Methods

Separation of CD34$^+$ cells

After obtaining informed consent from the patient a bone marrow aspirate was performed. The CD34$^+$ cell population was selected from mononuclear cells using immunomagnetic column separation techniques (Miltenyi Biotec, Sunnyvale, CA, USA) as according to the manufacturer's specifications. Purity following two passes over the immunomagnetic columns was above 90%.

Post-transplant engraftment and chimerism studies

Molecular genetic methods were used to assess quantitative fraction of donor *vs* recipient hematopoiesis in serial samples of bone marrow (BM) obtained after engraftment. Genomic DNA was obtained from patient and donor prior to transplant and analyzed by polymerase chain reaction amplification of the minisatellite genetic marker D1S80.[11] In the post-transplant period, samples of patient bone marrow or blood were tested for the same markers to assess the origin of cells as either donor or recipient. These data are calculated as percent donor engraftment and reported in quartile increments or as a precise percentage of donor cell engraftment.

Cytogenetics

Cytogenetic evaluation included G-banded analysis of a minimum of 20 metaphase cells, from unstimulated, cultured (24 h) bone marrow aspirates. All numerical and structural chromosomal abnormalities were characterized according to the ISCN, 1995.[13]

Case report

In 1989, the patient presented, aged 34, with fevers and lymphocytosis. The diagnosis of chronic lymphocytic leukemia (CLL), stage IV, was made. He was treated with chlorambucil, prednisone, and pentostatin according to ECOG protocol 1488, and achieved a complete response after 9 months of treatment. In 1993, he had disease progression with pleural effusion and abdominal adenopathy, associated with idiopathic thrombocytopenic purpura (ITP) that responded poorly to cladribine (2-CDA). A splenectomy was performed resolving the ITP and he required no further therapy. In January 1995, the CLL progressed again and he was restarted on his original chemotherapy regimen of chlorambucil, prednisone, and pentostatin, but continued to have bulky retroperitoneal disease after 9 months of therapy. Therapy was switched to cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP), and he was referred for HSCT for refractory CLL. Unexpectedly, the bone marrow biopsy performed at the time of his evaluation for HSCT showed, besides CLL, morphological and cytogenetic evidence of t-MDS with two clones, one with monosomy 7 (Figure 1a) and the other with deletion of 20q (Figure 1b). After a total of three cycles of CHOP for CLL cytoreduction he underwent an HLA-matched related donor HSCT from his brother in September of 1996. The preparative regimen included cyclophosphamide 60 mg/kg, on 2 sequential days, and 1320 cGy of total body irradiation in eight fractions. Graft-versus-host disease prophylaxis consisted of cyclosporin A (CsA) and methotrexate. The immediate post-transplant course was complicated by neutropenic fevers and hyperbilirubinemia. There was no evidence of acute GVHD, but 3 months post transplant he developed chronic GVHD which was managed with steroids and CsA. Two months later he developed CMV viremia that responded to a course of ganciclovir. He was known to be CMV positive before HSCT. Chronic GVHD resolved 5 months later, and all immunosuppressive therapy was stopped in January 1998.

Throughout his post-transplant course the patient had a persistently elevated white cell count (WBC), ranging from 8 to 28 $\times$ 10^9/l. The differential count invariably showed increased neutrophils. In addition he remained thrombocytopenic (range 40 to 88 $\times$ 10^9/l). Several bone marrow biopsies were obtained to rule out MDS relapse. Bone marrow cellularity ranged between 40 and 65%, with trilineage engraftment with adequate numbers of megakaryocytes. Morphology and cytogenetics were repeatedly normal. Engraftment studies always showed 100% donor hematopoiesis (Table 1). In February 2000, 3 years post transplant, he was admitted to the hospital with respiratory symptoms and low grade fevers that resolved with broad-spectrum

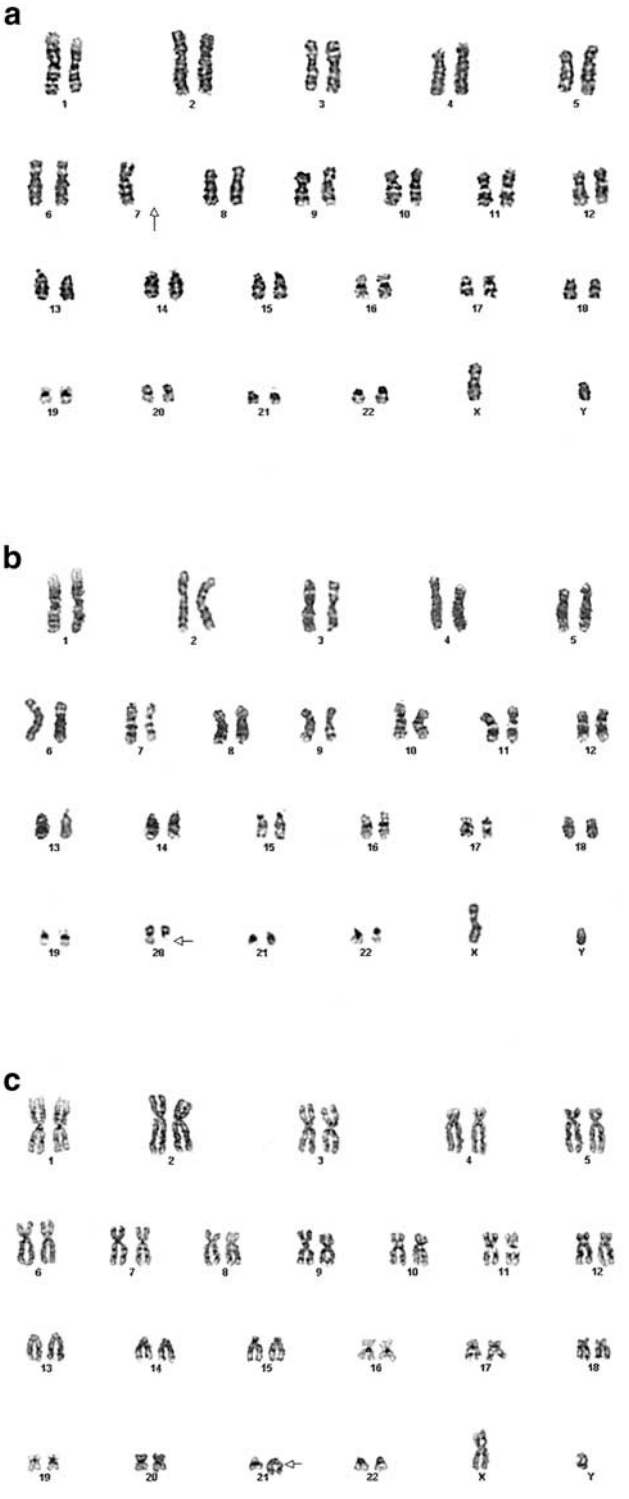

Figure 1 Figure 1 Cytogenetic studies performed before and after hematopoietic stem cell transplant showing chromosomal abnormalities compatible with t-MDS/AML. The two clones observed pre-hematopoietic stem cell transplant were **(a)** 5,XY,−7 and **(b)** 46,XY,del(20)(q11.2q13.3). **(c)** The clone observed in donor cells when the patient presented with MDS/AML showed 46,XY,add(21)(q22).

Table 1 Results of blood counts, engraftment, and cytogenetics studies performed after hematopoietic stem cell transplantation

Months post transplant	*Blood counts*		*Cytogenetic studies*[a]	*Engraftment studies*[b]	
	WBC	*ANC*		*Specimen*	*% Donor*
1	20.8×10^9/l	12 500	—	BM	96.3
6	8.0×10^9/l	5000	46, XY [20]	PB MNC	100
6	—	—		PB PMN	100
12	24.5×10^9/l	13 000	46, XY [20]	BM	100
24	23.8×10^9l	11 180	46, XY [50]	BM	100
36	17.6×10^9/l	10 400	46, XY [20]	BM	100
42	12.8×10^9/l	8200	46, XY, add(21)(q22)[3]	BM	100
42	—	—	46, XY [27]	BM $CD34^+$ cells[c]	98.6

WBC = white blood cells; ANC = absolute neutrophil count; BM = bone marrow; PB = peripheral blood; MNC = mononuclear cells; PMN = polymorphonuclear cells.
[a]Cytogenetic studies performed by G-banding techniques.
[b]Engraftment studies performed by PCR amplification of the minisatellite genetic marker D1S80.
[c]$CD34^+$ cells were purified from the bone marrow by immunomagnetic labeling techniques.

antibiotics. After discharge, CMV was identified in the bronchial lavage culture and he was again treated with ganciclovir. At that time 2% blasts were noticed in the peripheral blood. Another bone marrow biopsy was obtained. This time, the bone marrow was hypercellular (80 to 85% cellular) with 18% blasts with pronounced megakaryocytic dysplasia. Immunophenotypic evaluation showed that the blasts were CD34, CD13, CD33, CD36 and CD11c positive. Engraftment studies found 100% donor hematopoiesis. Cytogenetic studies, revealed a clone, comprising 10% (three of 30 metaphases), characterized by additional material of unknown origin replacing the distal long arm of one chromosome 21 at q22 (Figure 1c). This chromosomal abnormality was different from the cytogenetic abnormalities observed in the pre-transplant host t-MDS clone. CD34-positive cells were purified from the patient's peripheral blood and engraftment studies were performed again, confirming 100% donor origin of the blasts (Figure 2a–c). Once the diagnosis of donor cell MDS was made, a bone marrow biopsy was performed on the donor to evaluate it for similar cytogenetic changes and evidence of MDS. Morphology and cytogenetics were normal.

The patient was treated with idarubicin (12 mg/m^2 days 1 and 2) and cytarabine (100 mg/m^2 days 1 to 5). His induction course was complicated by a catheter-related right upper extremity deep vein thrombosis for which he was started on heparin, febrile neutropenia, and upper gastrointestinal bleeding. Following recovery of an absolute neutrophil count above 1000 a repeat bone marrow biopsy showed persistent leukemia. He developed diffuse alveolar hemorrhage, retroperitoneal bleeding, and metabolic complications with renal and hepatic failure that eventually led to his death.

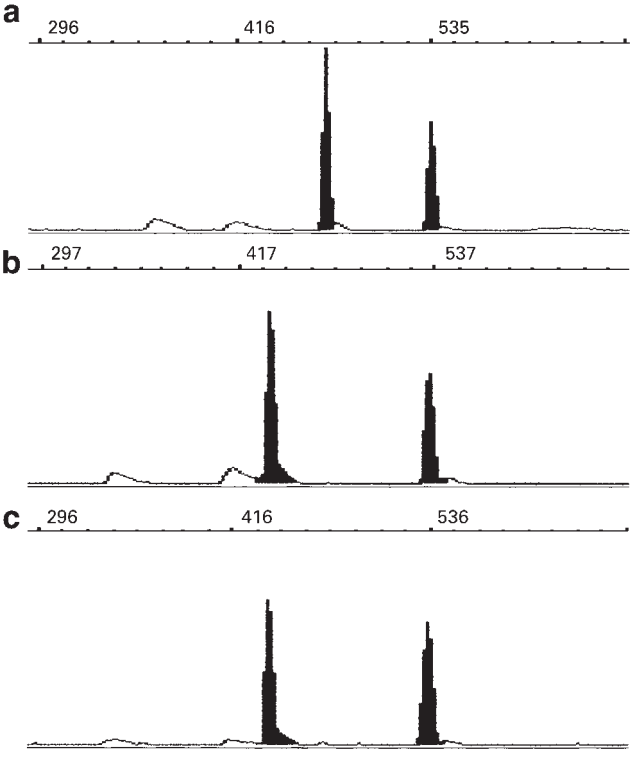

Figure 2 Figure 2 Eletropheretogram analysis of the pretransplant recipient **(a)** and donor **(b)** DNA samples. The analysis by PCR amplification of the minisatellite genetic marker D1S80 showing one unique and one common allele for the donor/recipient pair. **(c)** Post-transplant sample of leukemic $CD34^+$ cells showing 98.6% of donor-derived alleles.

Discussion

The increased risk of developing MDS/AML is well recognized in patients who receive cytotoxic treatment with alkylating agents and/or radiation therapy with or without autologous HSCT support.[1,2,14–16] Prolonged exposure to alkylating agents for CLL therapy may have played a role in the initial development of MDS in our patient. In addition, cladribine has also been implicated in the development of therapy-induced MDS.[17] More than 70% of patients with MDS/AML secondary to alkylating agent therapy have abnormalities on chromosomes 5 and/or 7.[2] In our patient, the chromosomal abnormalities observed before HSCT suggest therapy-related MDS, but *de novo* MDS can not be ruled out. Cytogenetics on the blasts of his post-transplant MDS/AML found additional material on chromosome 21q22, but no abnormalities on chromosomes

7 and 20. This new clone had not been observed in previous examinations. Abnormalities at chromosome 21q22 have been reported in therapy-induced MDS/AML most often associated with topoisomerase II inhibitors, such as anthracyclines,[3,4] that this patient received.

Donor cell leukemia after allogeneic HSCT has been reported sporadically since the early 1970s.[9,12,18–20] Cytogenetic studies evaluating gender mismatched donor/recipient pairs and/or polymorphism were used to diagnose donor cell leukemia in the cases reported in the 1970s and 1980s.[9,18,19] Molecular engraftment studies, initially based on restriction fragment length polymorphism,[21] and more recently characterized by minisatellite polymorphisms, provide a highly sensitive and accurate test for donor cell engraftment, and have been used in the more recent reports describing donor cell leukemia.[12] In the present case, molecular engraftment studies demonstrated full chimerism even when only CD34-positive cells were studied, confirming donor-derived hematopoiesis and associated MDS/AML as the blast population was CD34 positive.

As the donor had not been exposed to cytotoxic therapy, it raised the question whether the donor could have had incipient *de novo* MDS not detected in the pre-transplant evaluation. Hematopoietic reconstitution after allogeneic HCST by donor cell MDS[22] and acute myeloid leukemia[23] have been reported. However, morphological and cytogenetic evaluation of the donor's bone marrow, at the time the patient presented with donor cell MDS, did not find any evidence of MDS or AML.

The pathogenesis of leukemia developing in donor cells is not clear. Normal interactions between marrow stroma and hematopoietic progenitors are required for adequate marrow function.[24] In our patient, post-transplant bone marrow biopsies repeatedly showed tri-lineage hematopoiesis, but the peripheral blood had a persistently increased WBC with neutrophilia, and thrombocytopenia, suggesting abnormal hematopoiesis. Although speculative, it is possible that inherited or chemotherapy-induced microenvironment abnormalities caused defective signaling with consequent ineffective hematopoiesis, which ultimately led to the development of MDS/AML.[25] Most studies demonstrate that marrow stromal cells remain of host origin after allogeneic HSCT.[26] Therefore, chemotherapy or leukemia-induced recipient stromal abnormalities might contribute to the malignant transformation of donor progenitor cells.[12,19,24] *In vivo* and *in vitro* models suggest that a stromal lesion can be the first step in the disease process and the development of the hematological malignancy a secondary event.[24] In the present case, it is therefore possible that a primary stromal defect, caused by chemotherapy exposure or caused by the presence of CLL, caused the development of MDS before and after HSCT.

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