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Unrelated hematopoietic stem cell transplantation for Cernunnos-XLF deficiency

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Abstract: Cernunnos-XLF deficiency is a rare CI characterized by a defective DNA DSB repair mechanism. Its clinical manifestations are growth retardation, dysmorphic features, malformations, and severe B- and T-cell lymphopenia. BM failure may complicate the clinical picture. To date, there have been no described patients with CSy undergoing allogeneic HSCT. We report a case of CSy treated successfully with unrelated allogeneic HSCT after a reduced-intensity conditioning regimen. Two yr after HSCT, the patient maintains full donor engraftment, normal hematopoiesis, and progressively improving immune competence, thus suggesting that HSCT may be the treatment of choice for CSy.

Maura Faraci[1]*, Edoardo Lanino[1]*, Concetta Micalizzi[1], Giuseppe Morreale[1], Daniela Di Martino[1], Laura Banov[1], Patrizia Comoli[2], Franco Locatelli[2], Annarosa Soresina[3], Alessandro Plebani[3]

[1]Department of Hematology–Oncology and Hematopoietic Stem Cell Unit, G. Gaslini Children Research Institute, Genoa, Italy, [2]Laboratory of Transplantation Immunology and Pediatric Hematology/Oncology, Fondazione IRCSS Policlinico San Matteo, University of Pavia, Pavia, Italy, [3]Department of Pediatrics and Institute of Molecular Medicine "A. Nocivelli," University of Brescia, Bresica, Italy

Key words: Cernunnos syndrome – hematopoietic stem cell transplantation – Epstein–Barr virus–post-transplantation lymphoproliferative disease – congenital immunodeficiency

Dr. Edoardo Lanino, Department of Hematology–Oncology and Hematopoietic Stem Cell Unit, G. Gaslini Children Research Institute, Largo Gaslini 5, 16148 Genoa, Italy
Tel.: +39-010-5636405
Fax: +39-010-3777133
E-mail: edoardolanino@ospedale-gaslini.ge.it

*Both the authors equally contributed to the work.

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Abbreviations: *a.n.*, absolute number, aGvHD, acute GvHD; ANC, absolute neutrophil count; ATG, anti-thymocyte globulin; BM, bone marrow; cGvHD, chronic GvHD; CI, congenital immunodeficiency; CMV/EBV, cytomegalovirus/Epstein–Barr virus; CSA, cyclosporine A; CSy, Cernunnos syndrome; CTL, cytotoxic T lymphocytes; CY, cyclophosphamide; DEB, diepoxybutane; DSBs, double-strand breaks; G-CSF, granulocyte colony-stimulating factor; GvHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; LY, lymphocytes; MPD, methylprednisolone; MTX, methotrexate; *n.v.*, normal value; NHEJ, non-homologous end joining; NK cells, natural killer cells; PB, peripheral blood; PBMC, peripheral blood mononuclear cell; PLT, platelets; PTLD, post-transplantation lymphoproliferative disease; RIC, reduced-intensity conditioning; STR–PCR, short tandem repeat–polymerase chain reaction; XLF, XRCC4-like factor.

Cernunnos-XLF deficiency is a rare CI with a defective DNA DSB repair mechanism. DNA DSBs are among the most toxic DNA lesions caused by intrinsic sources (replication errors or DNA-damaging agents) or extrinsic factors as ionizing radiation (1). In Cernunnos-XLF, the result of defect of DNA DSB repair mechanism is a mutation of the Cernunnos-XLF gene (1, 2). This gene encodes for a component of the DNA repair machinery, which acts on the DNA breaks produced during V(D)J and immunoglobulin class switch recombination processes. This occurs through a NHEJ pathway, hence the immunodeficiency that is observed in this disorder. To date, only five patients have been reported with this genetic defect, and all of them

were found to have increased cellular sensitivity to radiation (1).

The main clinical features characterizing Cernunnos-defective patients are growth retardation, microcephaly, dysmorphic features (i.e., bird-like face), bone and urogenital malformations, and immunodeficiency. Immunodeficiency is characterized by mild-to-severe B- and T-cell lymphopenia, whereas NK cells are unaffected. The T-LY subsets are essentially composed of memory T cells with impaired function, as determined by low *in vitro* mitogen-induced T-cell proliferation. The clinical and immunological phenotype of these patients resemble that observed in patients with ligase IV deficiency and Nijmegen breakage syndrome, both of which are characterized by defects of the genes encoding for proteins contributing to the NHEJ repair pathway (3).

Due to their immunodeficient state, Cernunnos-deficient patients suffer from recurrent bacterial and viral infections. Treatment is mainly supportive, and immunoglobulin replacement therapy has been suggested to be the treatment of choice.

Some of these patients develop autoimmune anemia, thrombocytopenia, and BM aplasia. To date, only three of the five reported patients are still alive (1), thus emphasizing the severity of this disease and suggesting the need for more aggressive and definitive therapeutic approaches.

This report describes the outcome of an Italian patient with CSy, who was the first ever successfully undergo allogeneic peripheral HSCT with the purpose of curing the disease.

Case report

We describe a case of a 12-yr-old male, born at the 37th wk of gestation from third-degree consanguineous parents. Intrauterine growth had been delayed, resulting in low birth weight (1670 g; 2 s.d. below the mean), reduced length (46 cm; 2 s.d. below the mean), and reduced head circumference (33.5 cm; 2 s.d. below the mean). During the neonatal period, a diagnosis of Shwachman syndrome was hypothesized on the basis of growth delay and the presence of macrocytic anemia and neutropenia.

The patient was brought to our attention at the age of 12 months, because of recurrent respiratory tract infections and failure to thrive. His weight, height, and head circumference were under the third percentile and he had a bird-like face. Blood counts demonstrated only a moderate anemia (hemoglobin levels 9.9 g/dL). BM aspirate was normal. Immunological work up showed low serum levels of IgG (60 mg/dL, *n.v.* 700–1600 mg/dL) and IgA (< 6 mg/dL, *n.v.* 70–400 mg/dL), and normal IgM levels (104 mg/dL, *n.v.* 40–230 mg/dL), with absent antibody response to tetanus toxoid vaccination. Cytofluorimetric analysis of LY subsets gave the following results: 44% CD3+ (*a.n.* 455/cmm); 31% CD4+ (*a.n.* 320/cmm); 19% CD8+ (*a.n.* 195/cmm); 4% CD19+ (*a.n.* 40/cmm). Mitogen-induced lymphoproliferative response was slightly reduced when compared with that of a healthy control (data not shown). A diagnosis of hypogammaglobulinemia with growth delay and dysmorphic features was made and immunoglobulin replacement therapy was regularly administered.

During the first two-yr follow up, the patient showed good control of infectious episodes, although with the persistence of macrocytic anemia, whereas ANC fluctuated. IgM serum levels progressively decreased from 104 to 11 mg/dL. At the age of three yr, he developed thrombocytopenia (PLT 81 000 $\times 10^9$/L). Marrow trephine showed hypercellularity with trilineage dysplasia, with the absence of blasts. During the following five yr, peripheral pancytopenia developed and the patient became transfusion dependent. Neutropenia was unresponsive to G-CSF and the patient suffered of recurrent lower respiratory tract infections.

Cellular sensitivity to radiation was documented as previously described (1). This finding, together with the clinical and immunological phenotype, was indicative of a defect of DNA repair mechanisms. However, sequence analysis of ligase IV and NBS1 genes, and the DEB test were normal.

At the age of nine yr, the patient's molecular defect was identified as a homozygous C259G change in exon 2 of the Cernunnos gene, resulting in R57G substitution (1). Considering the patient's severe clinical picture, which included recurrent respiratory opportunistic infections, chronic enteropathy with severe malnutrition and transfusion dependency, as well as the lack of an HLA-matched family donor, HSCT from a volunteer donor was considered and it was performed at the age of 10 yr. BM aspirate performed before HSCT revealed stable BM hypoplasia with a 30% cellularity and trilineage dysplasia, in the absence of blast cells and chromosomal abnormalities.

An unrelated donor (HLA-A, -B, and DRB1 identical at the allelic level and with a single disparity at locus C) was identified. The donor's CMV/EBV serological status was negative, while

the recipient was CMV/EBV positive. The RIC regimen included fludarabine (30 mg/m^2/day from day −6 to day −3) and CY (300 mg/m^2/day from day −6 to day −3) (4). GvHD prophylaxis consisted ATG (Thymoglobulin; Genzyme, Cambridge, MA, USA 3.75 mg/kg/day from day −6 to day −3), CSA (2 mg/kg/day from day −6) and short course MTX (10 mg/m^2 day +1; 8 mg/m^2 on days +3 and +6). The patient received an unmanipulated PB graft, containing 42.4×10^6/kg CD34+ cells, 130.5×10^7/kg CD3+ LY, and 17.6×10^7/kg CD19+ cells.

ANC and PLT engraftment was achieved on days +10 and +13, respectively. Twenty-six days after HSCT, the patient was discharged in healthy conditions without signs of aGvHD. Forty days after HSCT, the patient developed grade 2 skin aGvHD, responsive to MPD (2 mg/kg/day). On day +49, STR–PCR analysis on PB and BM showed complete chimerism (100% of donor cells), which was maintained throughout the whole post-transplant course.

Concurrently, multiple superficial lymphoadenopathies developed, associated with spleen and liver enlargement, and fever. PCR detection of EBV DNA demonstrated a dramatic increase in EBV copies in PB (12 884 250 EBV copies/1 mL blood and 745 357 EBV copies/100 000 PBMCs with a consensual increase of LY (*a.n.* 947/cmm), 50.8% of them being represented by CD20+ cells (*a.n.* 482/cmm). A diagnosis of EBV–PTLD was made and donor origin of CD20+ cells was confirmed by STR. Despite two standard courses of anti-CD20 monoclonal antibody (rituximab; 375 mg/m^2/dose for four weekly doses), which led to a significant decrease in EBV–DNA copies (minimum level 8850 EBV copies/100 000 PBMC), the disease progressed. Multiple nodular lesions developed in the liver and lungs, the paranasal and mastoid sinuses, and the hypopharynx also became involved. Subsequent administration of reduced dosage chemotherapy according to the CHOP protocol (5) (prednisone: 20 mg/m^2 for two days and 40 mg/m^2 for three days; CY: 200 mg/m^2 day +2 and 600 mg/m^2 day +6; vincristine: 1.35 mg/ m^2 day +6; adriamycin: 35 mg/m^2 day +6; and G-SCF stimulating factor: 5 μg/kg from day +9), combined with additional doses of rituximab, led to the stabilization of EBV-related lesions, which were finally cleared through four infusions of EBV-specific CTL obtained by his original donor (median dose: 1×10^6 cells/kg body weight), which had been prepared accordingly to a previously reported method (5). Chemotherapy administration was followed by severe mucosal and hematological toxicity. The patient developed a moderate cGvHD responsive to low dose of steroids and CSA, these immunosuppressive treatments were discontinued 21 and 23 months after HSCT, respectively, to speed the specific antiviral T-cell-mediated immune reconstitution. At present, 12 months after the last CTL infusion, and 26 months after HSCT, the patient is in complete clinical and instrumental remission of PTLD and STR–PCR confirmed a full donor engraftment on PB and BM. The patient is currently receiving monthly immunoglobulin replacement therapy as a consequence of prolonged therapy with rituximab (total 16 doses).

He is in good health (Lansky score 100%), with the absence of infective episodes. The LY subsets are within the normal range except a low value of B-LY related to prolonged therapy with rituximab [78% CD3+ (*a.n.* 2162/cmm); 31% CD4+ (*a.n.* 859/cmm); 42.2% CD8+ (*a.n.* 1169/cmm); 7.1% CD19+ (*a.n.* 196/cmm)]. The complete immunological reconstitution is summarized in Fig. 1.

Discussion

Allogeneic HSCT is the treatment of choice for many life-threatening, combined, and primary immunodeficiencies (CI) (6). To the best of our knowledge, this is the first description of successful HSCT in a child affected by a recently identified variant of primary immunodeficiency caused by Cernunnos-XLF gene defect. This immunodeficiency is caused by a mutation of a gene encoding for a protein, which plays a pivotal role in the repair of DNA DSBs via the NHEJ pathway. DNA DSBs represent a serious form of DNA damage, potentially leading to replication errors, loss of genomic material, and eventually, cell death or carcinogenesis (7). This may explain the various clinical features of patients affected by CSy, in whom immunodeficiency is part of a more complex clinical phenotype characterized by growth delay, hematological abnormalities, and syndromic features (1).The clinical and genotypic characteristics of CSy are reminiscent of those of ligase IV deficiency, Nijmegen Breakage syndrome, or Fanconi disease.

We report the case of a patient affected by CSy, who presented early hypogammaglobulinemia (requiring regular immunoglobulin replacement therapy) and who developed over time progressive BM failure, with trilinear dysplasia unresponsive to treatment. The BM pattern of CSy mimics that observed in Fanconi disease, for which HSCT is strongly recommended (8). In the absence of an

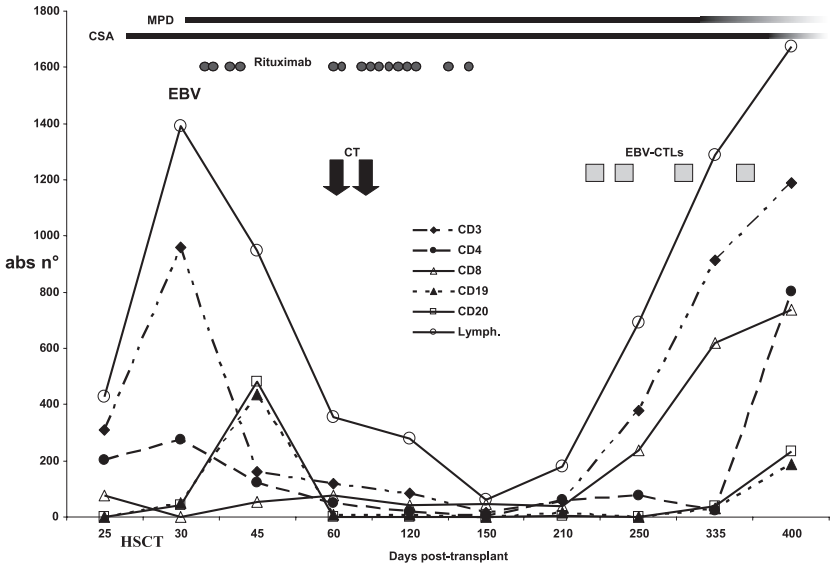

Fig. 1. Post-HSCT immunological reconstitution. abs, absolute number; CSA, cyclosporine A; MPD, methylprednisolone; EBV, Epstein Barr virus; EBV-CTLs, EBV-specific cytotoxic T lymphocytes; HSCT, hematopoietic stem cell transplantation; Lymph., lymphocytes; CT, chemotherapy.

HLA-identical family donor, the use of a matched unrelated donor is for a valuable option for offering HSCT to patients affected by CI (9). However, many patients affected by these disorders have significant comorbidities. The use of RIC regimens may be better tolerated, thus improving patient survival and decreasing the acute and chronic toxic effects that are usually observed after conventional myeloablative regimens (10). In our case, RIC was followed by mild acute toxicity, while the use of PB as a cell source possibly contributed to the development of cGvHD, requiring prolonged immunosuppressive therapy.

Full donor chimerism was documented early after transplantation with STR–PCR analysis performed on PB and BM, and was regularly confirmed during the follow-up period.

The clinical course of our patient was complicated by the development of EBV–PTLD with multiple large solid nodules in liver/spleen/lungs and enlargement of multiple lymph nodes. It has already been observed that the use of RIC increases the risk of EBV reactivation as results of pronged lymphopenia due to immunosuppression (11). Veys et al. (10) reported an unexpected increase in EBV reactivations when compared with conventional procedures in 81 children affected by CI who received HSCT after RIC. Moreover, Toita et al. (12) described a case of a young patient with DNA ligase IV syndrome who developed non-Hodgkin large diffuse B-cell lymphoma. These authors speculated that there was a correlation between the ligase IV syndrome and the occurrence of malignancies.

In our case, we hypothesize that the occurrence of EBV–PTLD may have a multifactor origin. In particular, the use of RIC and high-dose ATG prior to HSCT, as well as the use of an EBV negative donor in a recipient EBV positive, could explain the clinical peculiarities and the prolonged course of EBV–PTLD lymphoma that we observed in this patient (13).

Therapy for EBV–PTLD still yields unsatisfactory results, and the incidence of mortality remains high (14). As in our patient, EBV–PTLD was not responsive to rituximab, chemotherapy and EBV-CTL infusions, from the original donor, were employed, based on the data concerning treatment of EBV-related PTLD occurring in recipients of renal transplantation (5). Severe, chemotherapy-derived acute toxicity was possibly attributable to the early HSCT period and to the high sensitivity to chemotherapy that characterizes defective DNA DSB repair mechanism syndromes, such as CSy. Discontinuing immunosuppressive therapies (MPD and CSA) after cGvHD resolution played an important role in facilitating immune reconstitution and, consequently, in preventing recurrence of EBV–PTLD.

HSCT from an unrelated donor may represent the therapy of choice for Cernunnos patients who develop BM failure. In diseases with DNA repair defects, RIC is recommended to decrease transplant-related toxicity and mortality. In this setting of patients, the choice of an EBV-positive donor may be preferable to decrease the incidence of EBV–PTLD. Moreover, as the incidence of secondary malignancies is more frequent in patients who received HSCT to cure DSBs syndromes (as Fanconi anemia), we suggest a careful follow-up for this late complication also in this rare category of patients affected by CSy.

Acknowledgments

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