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SPECIAL REPORT

First experience of hematopoietic stem cell transplantation treatment of Shwachman–Diamond syndrome using unaffected HLA–matched sibling donor produced through preimplantation HLA typing

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The only proven cure for Shwachman–Diamond syndrome (SDS) bone marrow failure is allogeneic hematopoietic stem cell transplantation (HSCT). However HSCT with donors other than HLA-identical siblings is associated with high mortality and unfavorable prognosis. This paper presents the first experience of HSCT treatment of SDS using an unaffected HLA-identical sibling produced through preimplantation genetic diagnosis (PGD). The patient was a 6-year-old blood transfusion-dependent SDS baby girl with secondary myelodysplastic syndrome, for whom no HLA-identical donor was available. As a result of PGD, two unaffected HLA matched embryos were identified; one of them was randomly selected for transfer, resulting in a clinical pregnancy and birth of an apparently healthy child. The patient underwent allogeneic transplantation of cord blood hematopoietic stem cells, together with bone marrow from this sibling, resulting in complete hemopoietic recovery. The patient was no longer transfusion-dependent and had normal blood values 160 days after transplantation.

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INTRODUCTION

Shwachman–Diamond Syndrome (SDS) is a rare autosomal recessive multisystem disorder characterized by exocrine pancreatic insufficiency, progressive bone marrow failure, and predisposition to myelodysplasia and leukemia.[1] The only proven long-term cure for bone marrow insufficiency and myelodysplastic syndrome (MDS) in SDS is allogeneic hematopoietic stem cell transplantation (HSCT).[2] HSCT in SDS is associated with a high risk of transplantation-related complications, including cardiotoxicity, GvHD and opportunistic infections. The best results can be achieved only by HSCT from HLA-identical sibling donors, unavailable for most SDS patients.[3–6] When no HLA-compatible sibling is available, couples have an option of preimplantation genetic diagnosis (PGD) to preselect unaffected HLA-matched embryos for transfer, to produce a potential sibling donor, not only free of SDS but also HLA compatible with the proband, and to ensure the success of bone marrow transplantation treatment.[7,8]

This paper presents the clinical case of a 6.9-year-old girl with SDS-associated bone marrow failure and secondary MDS who required an HLA-identical donor.

SUBJECTS AND METHODS

History

The patient was anemic at birth and developed neutropenia, generalized atopic dermatitis and stridor. At the age of 3 months, the girl was admitted to the R. Gorbacheva Memorial Research Institute for Pediatric Oncology, Hematology and Transplantation with suspected MDS. The clinical studies showed severe anemia, decrease of lipase and pancreatic amylase levels, cytolytic syndrome, steatorrhea, and decrease of fecal elastase in a coprogram. Based on clinical features, family history and laboratory studies, SDS was suspected, but molecular genetic testing was not performed at this time. At 6 months, myelogram and bone marrow biopsy showed hypocellularity, considerable restriction of granulocyte and erythroid lineages, and multilineage dysplasia with an excess of blasts (15–15.6%). Cytogenetic analysis showed normal karyotype. The presence of bone marrow and exocrine pancreatic insufficiency (steatorrhea and decrease of pancreatic amylase), clinical features and medical history confirmed the diagnosis of SDS with signs of transformation to MDS (refractory anemia with excess blasts).

Within the next few months, the disease progressed with thrombocytopenia, agranulocytosis and infectious complications, including bilateral pneumonia and phaeohyphomycosis with lung involvement. Due to unavailability of a matched related donor or an unrelated donor in the international register, extending pancytopenia, signs of MDS transformation and recurrent infectious complications, the 16-month-old patient underwent HSCT from her father despite a 50% HLA match in 2010. In the post-transplantation period, the patient developed CMV disease with bone marrow involvement, and the graft was subsequently rejected (D+135). Hence the couple was offered an

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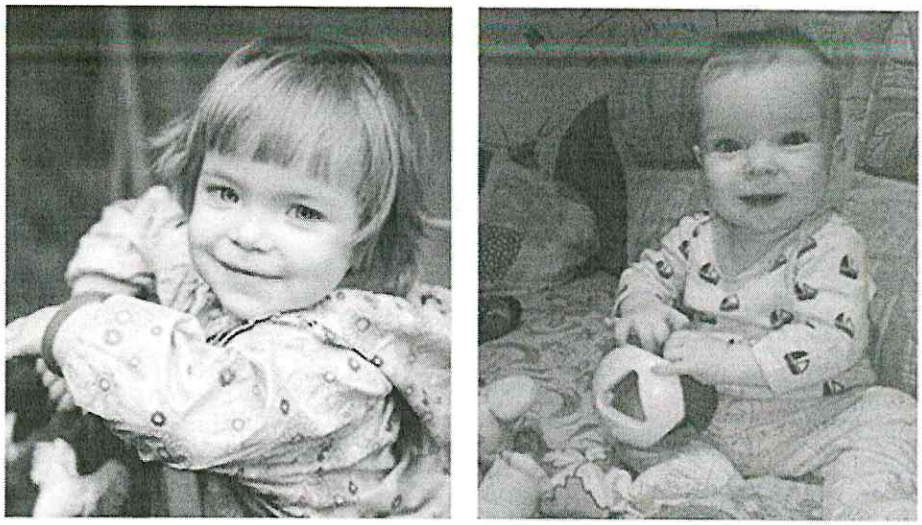

Figure 1. Patient A (6-year old girl) and healthy HLA-identical sibling Ar., produced by PGD.

option of PGD to produce an unaffected HLA-matched offspring as a source for HSCT.

In vitro fertilization and embryo selection by PGD

The molecular genetic testing with Sanger sequencing showed that the patient was compound-heterozygous for SBDS gene mutations, c.183-184TA > CT and c.258+2T > C, inherited from her parents. In 2013, after controlled ovarian hyperstimulation, 13 mature oocytes were recovered, resulting in 11 embryos, of which 8 were available for testing of the presence of SBDS mutations and HLA type. Only two of these embryos were predicted to be unaffected and HLA-identical, and one was transferred, resulting in an unaffected HLA-matched full-term pregnancy and birth of a healthy child (Figure 1).

Characteristics of the sibling umbilical cord blood and bone marrow transplant

The patient, at the age of 6.9 years, underwent a second allogeneic HSCT of cord blood and bone marrow from the fully matched healthy sibling, whose cord blood was recovered at birth (109 mL, a total cell count of 1.06×10^9, $CD34^+$cell count of 9.86×10^6, 91.2% viability). The genetic analysis of the sample confirmed the absence of SBDS gene mutations and HLA identity with the patient. At the myeloexfusion, the donor was 2 years old with a weight of 12.5 kg, recipient weight was 15 kg. The donor sibling underwent the standard procedure, with no sign of complications. The 56 mL graft consisted of cord blood and bone marrow cells with the following concentration per recipient body weight: nucleated cells per kg 2.5×10^8, $CD34^+$ per kg 3.85×10^6, $CD3^+$ 13.4% and $CD3^+$ per kg 2.4×10^7. On D0 the sibling donor cord blood and bone marrow were infused through a central venous catheter according to the standard procedure.[9]

The sibling's condition after bone marrow donation showed no complications.

Characteristics of the patient before the second HSCT

According to Karnovsky scale modified by Lansky, the patient was completely active, adequate, ECOG 0. Creatinine clearance was 126 mL/min. Cardiac function assessment by echocardiography was in the normal range with cardiac output of 70.5%. There was insignificant liver enlargement, with diffuse changes of liver and pancreas and no free liquid in peritoneal cavity. The clinical blood analysis showed pancytopenia (agranulocytosis, thrombocytopenia and severe anemia), so the transfusions of platelet and packed RBC were still required. According to the myelogram, bone marrow was hypocellular, without blast count increase, and karyotype was normal. Biopsy showed hypocellular bone marrow (adipose tissue volume of 85%), with features of dysplasia.

Conditioning regimen

Myeloablative reduced toxicity conditioning regimen included fludarabine 30 mg/m^2 per day, 5 doses; treosulfan 14 g/m^2 per day, 3 doses; thiotepa 5 mg/kg per day, 2 doses; thymoglobulin 2.5 mg/kg per day, 3 doses. cyclosporine A 3 mg/kg per day (since D − 1) and mycophenolatemofetil 1200 mg/m^2 per day (from D − 3) were given as GvHD prophylaxis. Then, due to the signs of neurotoxicity and arterial hypertension, cyclosporine A was replaced by sirolimus at the D+1 (1 mg/m^2 per day).

RESULTS

Engraftment and immune reconstitution

The recovery of the WBC to $> 1 \times 10^9$/L was observed on D+23, with neutrophils $> 0.5 \times 10^9$ on D+28, and platelets $> 100 \times 10^9$/L on D+62. On D+100 after HSCT, the full donor chimerism was recorded with the PCR/STR loci analysis method, showing not less than 99% of cells of the donor origin. Myelogram and bone marrow biopsy on D+130 showed the cell count corresponding to the age (hematopoietic tissue volume of 85–90%), with all hematopoietic lineages present, and a considerable expansion of the erythroid lineage (Figure 2). The only concern was a persistence of 2% of patient's $CD3^-$ lymphocytes, which may affect the patient's prognosis due to a possible risk of graft rejection. On D+180 bone marrow trephine biopsy showed normal cellularity without signs of myelodysplastic changes, cytogenetic analysis detected normal male karyotype 46 XY, STR PCR of bone marrow revealed full donor chimerism (> 97% of donor cells).

Clinical outcome and transplantation-related complications

The early post-transplant period was complicated with grade II oral mucositis and gastrointestinal toxicity (diarrhea and colitis), grade I skin toxicity, febrile neutropenia, tonsillitis (on D+83) and cervical lymphadenitis (*Acinetobacter baumanii*), which was successfully conservatively treated with antibiotics. The patient was discharged on D+106 post transplant and did well as an outpatient without any subsequent hospitalizations. On D+180 cardiac evaluation revealed a normal echocardiogram without any evidence of cardiac dysfunction. She remained quite short in stature and underweight (weight and height < 3th percentile for normal population, wt *Z*-score − 2.83 SD, height *Z*-score − 2.7 SD, BMI − 13.7, *Z*-score

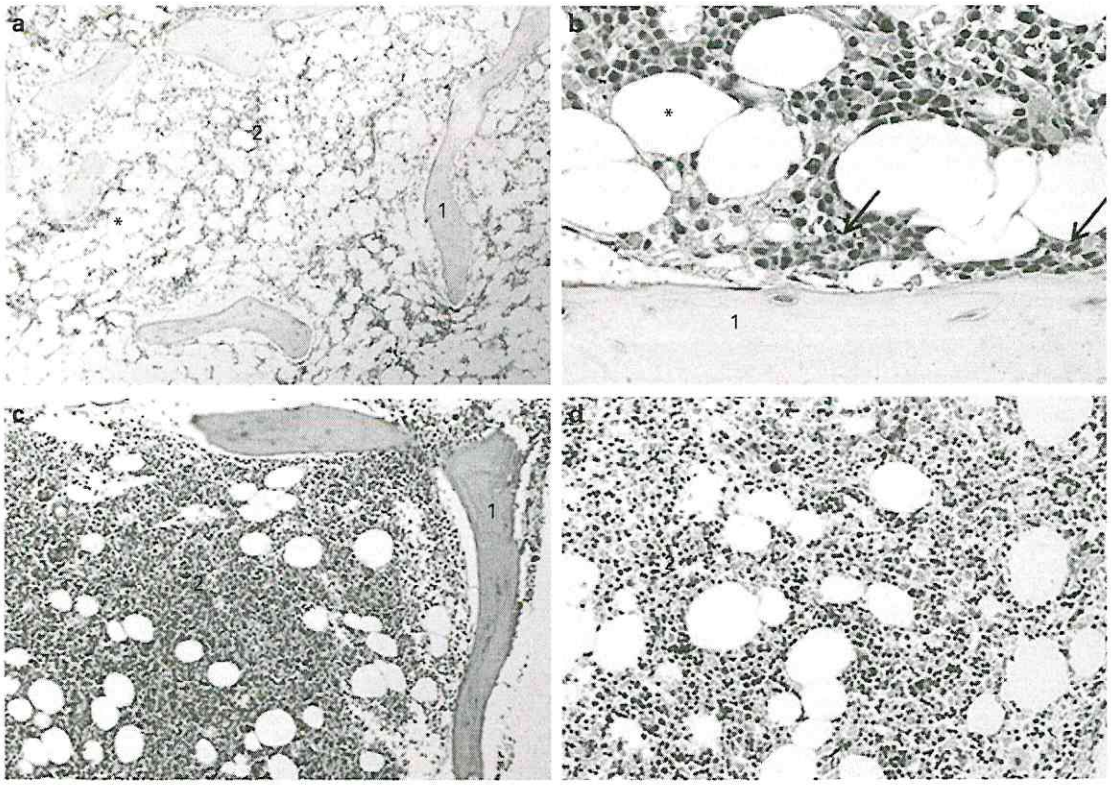

Figure 2. Trephine bone marrow biopsy: (**a**, **b**) prior HSCT from HLA-identical donor; (**c**, **d**) 120 days after HSCT. * – adipocytes; arrow – atypical paratrabecular localization of erythrocaryocytes, with dysplastic and megaloblastoid features of individual ones; 1 – bone trabeculae; 2 – hematopoietic tissue. (**a**–**c**) Hematoxylin and eosin; (**d**) azure and eosin. A ×40, B ×400, C ×100, D ×100.

– 1.4 SD) and needed substitution therapy and nutritional support with required enzymes and relevant fat-soluble vitamins.

On D+243 (20.12.2016) no signs of acute or chronic GvHD and other complications have been observed. She is transfusion independent, has normal CBC (WBC 5.7×10^9/L, ANC 3808/μL, hemoglobin 132 g/L, platelets 190×10^9/L) and a Lansky score of 100%.

DISCUSSION

PGD has become a realistic option for obtaining an unaffected HLA-identical source of HSCT treatment of congenital and acquired bone marrow failures, with no risk for a donor child, while providing a real chance for a sibling's rescue.[7,8,10] The objective of PGD for HLA typing to produce an HLA-matched donor is not only to benefit the affected sibling, as the couples are planning to have another healthy child anyway, which cannot be achieved without PGD.[11] Currently, hundreds of PGD cases have been performed for HLA matching, resulting in dozens of successful HSCTs from unaffected HLA-matched donors obtained by PGD.[7,8,12–14] The majority of the conditions treated by this approach were blood diseases, such as thalassemia and Fanconi anemia, but successful treatment cases were also reported for patients with acute lymphoblastic leukemia.[12]

Reported cases of SDS who have undergone HSCT[4,15] show that treatment success largely depends on the stage during which HSCT was performed, with 80% five-year survival in hematopoietic aplasia, and 30–40% survival with development of secondary MDS and signs of acute leukemia. The use of HSC from HLA-identical donors increases the general survival by 15–20%, and lowers rates of transplantation-related events. Considering that the patient had MDS signs with the tendency of transformation to leukemia, the use of HSCT from HLA-identical sibling was crucial and may have a significant impact on the prognosis. Thus, the reported experience of the first case of successful HSCT to the SDS patient from the HLA-identical healthy sibling derived through PGD demonstrates the usefulness of the approach for radical treatment of SDS. As described, the combined allogeneic HSC transplantation of cord blood and bone marrow allowed recovering the patient's hematopoiesis, no longer requiring regular blood transfusions.

CONFLICT OF INTEREST

AA Isaev is a share owner of PJSC Human Stem Cells Institute and its General Director; RV Deev, IL Plaksa, IV Potapov, EA Pomerantseva, AV Prikhodjko, AG Chogovadze and IY Bozo are employees of PJSC Human Stem Cells Institute. The remaining authors declare no conflict of interest.

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