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LETTER TO EDITOR

Combined haploidentical and umbilical cord blood transplantation for severe aplastic anemia: Unique hematopoietic reconstitution

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To the Editor:

Hematopoietic stem cell transplant (HSCT) with matched sibling donor (MSD) is considered the first line of therapy for patients with severe aplastic anemia (SAA) who are <40 years of age. Immunosuppressive therapy (IST) and alternative donor HSCT are other modalities of treatment [1]. Because of nonavailability of an MSD for two-thirds of the SAA patients and a significant rate of failure of IST, alternative donor HSCT has been tried with encouraging results [2]. In 2003, Fernández et al. [3] in a cohort of 11 patients with high-risk hematological malignancies successfully utilized a novel approach of supplementing cord blood unit (CBU) with $CD34^+$ cells from haploidentical donor to use the latter as a bridge for rapid neutrophil recovery followed

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by a persistent dominant CBU chimerism. In 2011, Gormley et al. [4] pioneered the use of haplo-cord transplant for SAA patients lacking histocompatibility leukocyte antigen (HLA)-compatible MSD/unrelated donor who failed IST. Typically, hematopoietic reconstitution after haplo-cord transplant shows a pattern of transient myeloid engraftment with haploidentical graft followed by dominant CBU chimerism [5]. Here, we report a case of haplo-cord HSCT for SAA that showed a unique hematopoietic reconstitution kinetics. The patient was found to have a dominant CBU peripheral blood (PB) chimerism (97%) on T + 12 with no evidence of haploidentical graft dominance at any time during the post-HSCT period.

Our patient is a 26-year-old male who presented in June 2012 with a 1-month history of fatigue and weakness. His initial complete blood count showed a hemoglobin of 4.5 g/dL, white blood cell count of 1.4×10^9/L, absolute neutrophil count of 0.4×10^9/L, and platelet count 2.0×10^9/L. Bone marrow biopsy showed a profoundly hypocellular (<5% cellularity) bone marrow consistent with SAA. Genetic testing showed a normal male karyotype without increased tendency of chromosomal breakage (normal diepoxy butane breakage analysis). Studies for bone marrow failure syndromes were negative for Diamond–Blackfan anemia and dyskeratosis congenita. Paroxysmal nocturnal hemoglobinuria panel showed a positive clone (2%). A telomere study performed in July 2015 showed shortened telomeres at the 9th percentile. HLA typing revealed that the patient did not have any MSD and a global search for a matched unrelated donor (MUD) was unsuccessful. He required red blood cell transfusion support and began IST with cyclosporine and antithymocyte globulin according to the standard guidelines [1]. He had a transient response to IST but relapsed after 14 months of completing IST. Trial of eltrombopag with a maximum daily dose of 150 mg was unsuccessful.

Because of failure of these therapies, a decision was made to administer HSCT. In preparation for his transplant, he was given rituximab 375 mg/m^2 on T−19 to decrease his risk of graft rejection/failure. He was also given intravenous (IV) immunoglobulin on T−8. Because of the unavailability of a suitable MSD/MUD, he underwent a combined haploidentical and CBU (haplo-cord) HSCT with conditioning regimen of fludarabine 25 mg/m^2/day IV for 5 days (T−6 to T−2), cyclophosphamide 60 mg/kg/day IV for 2 days (T−6 to T−5), equine antithymocyte globulin 40 mg/kg/day for 4 days (T−4 to T−1), and total body irradiation of 200 cGy on T−1. He received a granulocyte-colony-stimulating factor-mobilized and CD34$^+$-selected haploidentical donor graft from his cytomegalovirus-positive, ABO-compatible sister (recipient B+, donor B+). HLA typing for the patient, haploidentical sibling donor, and CBU is shown in Table 1. The patient was weakly positive for Class I antibody (against HLA A 11:03), which was found by a panel-reactive antibody (PRA) test prior to transplant. CD34$^+$ selection was done using a Miltenyi CliniMACS column (CA, USA). A total of 3.32×10^6 CD34 cells/kg were infused. No natural killer (NK) cells were present in the grafts of our patient. This was followed by infusion of 4/6 HLA-A-, HLA-B-matched CBU (2.1×10^7 total mononuclear cells/kg, 0.18×10^6/kg CD34$^+$ cells). Killer-cell immunoglobulin-like receptors (KIRs) ligand incompatibility for HLA-B was in the haplo → cord direction, whereas that for HLA C was in the graft → host direction. Graft-versus-host disease (GvHD) prophylaxis consisted of tacrolimus and mycophenolate mofetil (T + 1 to T + 14). Tacrolimus was started on T−3 to maintain goal trough of 8–12 ng/mL. It was gradually tapered and discontinued after 8 months.

PB chimerism studies by multiplex polymerase chain reaction on T + 13 showed 2% haploidentical related donor, 3% recipient, and 95% unrelated CBU DNA. Neutrophils engraftment (>500×10^9/L for 3 consecutive days) was attained on T + 22. His last platelet transfusion was on T + 25 after which he remained transfusion independent. Post-transplant neutrophil, platelet, and hemoglobin recovery are shown in Fig. 1A–C. PB chimerisms on T + 28, T + 52, T + 93, and T + 180 showed sustained CBU graft dominance with 97%, 98%, 98%, and 97%, respectively (Fig. 1D). In addition, T + 93 and T + 180 bone marrow chimerism showed 97% and 98% CBU graft, respectively (Fig. 1E). Complications included *Klebsiella pneumoniae* bacteremia, human herpesvirus-6, cytomegalovirus viremia, and adenoviremia, which were managed successfully according to standard guidelines. He remains well 20 months after HSCT with bone marrow evaluation at 1 year after transplant showing a 98% cord unit chimerism.

This patient's post-HSCT hematopoietic reconstitution pattern is unique due to several reasons. First, he exhibited a very unusual pattern of engraftment as compared with the one seen after most haplo-cord transplants. Unlike the typical pattern of transient haploidentical myeloid engraftment followed by persistent CBU engraftment, our patient had an unusually early dominant CBU chimerism, which remained stable throughout the follow-up period of 271 days post-transplant. Interestingly, this occurred despite a high degree of HLA mismatch (4/6) and KIR

Table 1 HLA typing of the patient, sister (haploidentical donor), and cord blood unit (CBU).

	A locus	B locus	C locus	DRB1 locus	DQB1 locus	DPB1 locus	HLA match
Patient	11:03	52:01	07:02	15:02	05:01	04:01	
	24:02	35:03	07:04	15:04	05:02	13:01	
Sister	02:01	40:06	15:02	15:01	06:01	04:01	5/10
	24:02	35:03	07:04	15:04	05:02	04:02	
CBU	11:03	52:01	07:03	15:02	05:01		4/6
	24:02	35:03	07:04	15:04	05:02		

Note. HLA = histocompatibility leukocyte antigen.

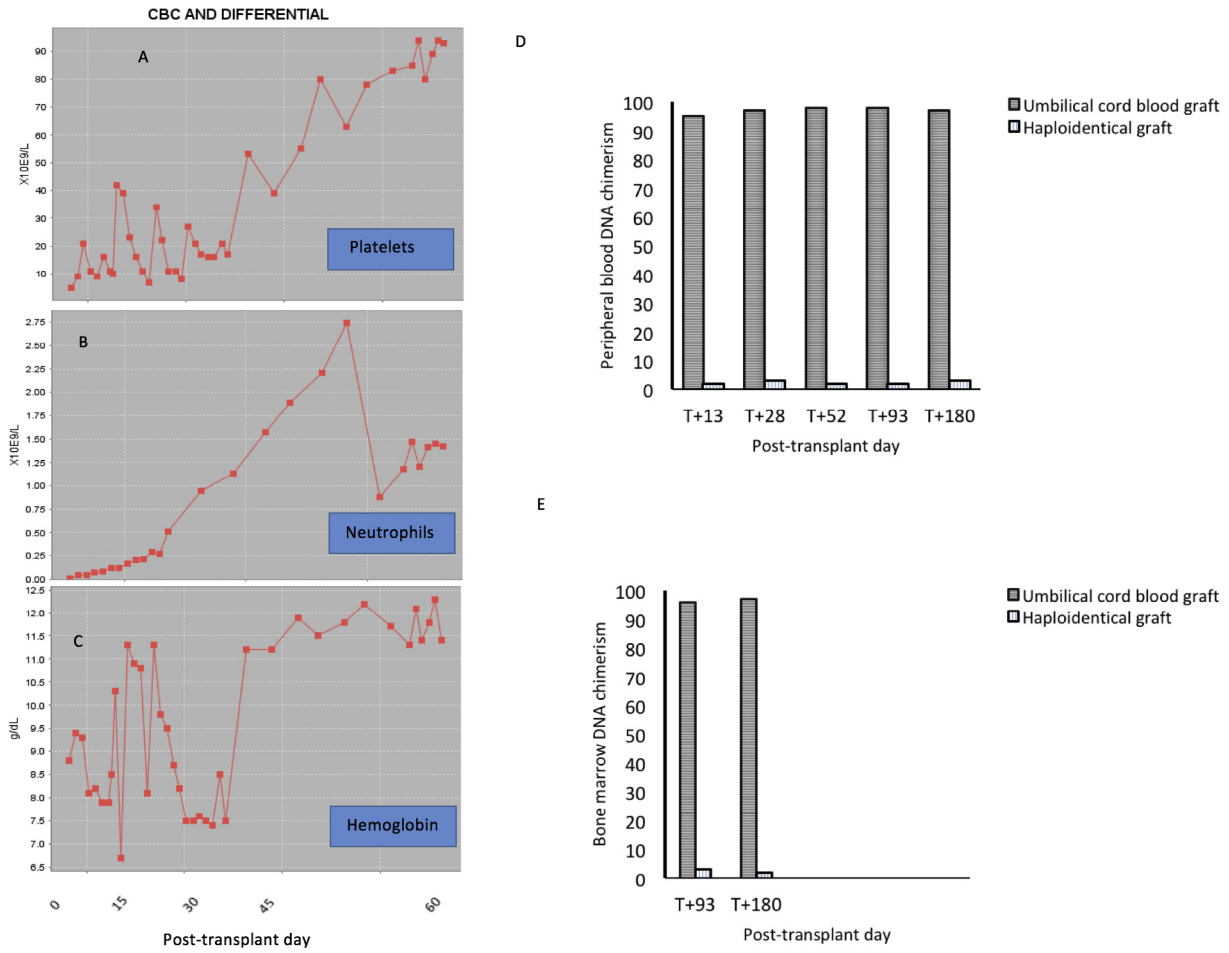

Fig. 1 Post-transplant hematopoietic reconstitution kinetics, (A) platelet recovery, (B) neutrophil recovery, (C) red blood cell recovery, with chimerism kinetics in (D) peripheral blood and (E) bone marrow for the patient. Chimerism values in both blood and bone marrow show a unique pattern of complete and persistent dominance of the umbilical cord graft throughout the reconstitution phase. CBC = complete blood count.

mismatch in the haplo → cord direction for the HLA epitope C, both of which are considered risk factors for failure of engraftment of CBU [6,7]. It is possible that the cord → haplo direction KIR mismatch at HLA epitope Bw4 in our patient lead to a more potent effect with elimination of the haplo graft. In addition, the inherent, hitherto unknown differences in the cellular and molecular composition of the haplo and cord grafts may have favored early cord engraftment with elimination of haplo graft. While the effect of total nucleated cells and $CD34^+$ cell numbers in the haplo and cord graft has been studied before, there are no reports on the effect of NK cell numbers in the graft [3—5]. Moreover, the presence of other unfavorable factors like the presence of a low PRA against HLA Class I antigen also did not lead to cord failure, despite a suboptimal $CD34^+$ dose from the CBU. Hence, our case illustrates the possibility of other unexplored factors involved in the determination of dominant graft following haplo-cord HSCT.

In 2011, researchers from the National Institutes of Health were the first to report on the use of haplo-cord transplant for patients with SAA refractory to IST who lacked an HLA-matched donor [4]. The same group recently reported on a randomized clinical trial of 47 patients with high-risk SAA refractory to IST who underwent haplo-cord (n = 28) versus MSD (n = 19) HSCT. Their study showed comparable 5-year overall survival after haplo-cord (91%) and MSD HSCT (87%) [8]. All the eight patients undergoing haplo-cord HSCT reported in the previous study were included in this report. We have summarized the results of all the patients in this study who underwent haplo-cord transplant along with our case in Table 2. To the best of our knowledge, there are no other reports on the use of haplo-cord HSCT for treatment of SAA. However, based on the excellent outcomes of these patients, haplo-cord HSCT appears to be a novel transplant methodology that can overcome the limitations of both the haploidentical and CBU HSCT, as has been previously suggested by van Besien and Childs [5]. The supplementation of CBU with the haploidentical stem cells lowers the cell dose requirement for the CBU, thereby expanding the donor pool for the recipients. It also shortens the duration of neutropenia by providing a transient myeloid bridge leading to early neutrophil engraftment with subsequent loss of haplo graft and dominance of the cord graft that possibly contributes to remarkable reduction in the incidence of GvHD. Atypical engraftment kinetics with ultimate haplo graft dominance is rarely seen.

Table 2 Review of haplo-cord hematopoietic stem cell transplants for severe aplastic anemia published in the literature.

Author (Year)	*n*	Median age in years (range)	Conditioning regimen	HLA matching of CBU	Cellular characteristics of grafts, median cells per kg (range)	GvHD prophylaxis	Median time to myeloid engraftment in days (range)	Total donor engraftment (engraftment pattern) at study endpoint	Graft failure	GvHD	Transfusion independence	Median follow-up	OS
Purev et al. [8]	28	20 (5—49)	Cy + Flu + eATG + TBI (200 cGy)	18: 4/6 10: ≥5/6	Haplo ○ CD34^{+a}, 3.2×10^6 ○ CD3^{+b}, 1.3×10^3 CBU ○ TNC^c, 3.6×10^7 (2.6—7.3) ○ CD34^{+a}, 1.5×10^5 (0.4—3.2)	Tacrolimus + MMF	Haplo ○ Neutrophil, 10 (6—28) ○ Platelet, 28 CBU ○ Neutrophil, 42 (10—18) ○ Platelet, NR	21/21 (100%) (18 UCB, 2 haplo, 1 none, salvaged with DUCBT)	1/21	Acute ○ Gr II—IV: 38% ○ Gr III—IV: 4% Chronic ○ Limited: 45% ○ Extensive: 8%	100%	40 mo	91% at 5 y
Our patient, 2018	1	26	Cy + Flu + eATG + TBI (200 cGy)	4/6	Haplo- ○ CD34^{+a}, 3.32×10^6 ○ CD3^{+b}, NR CBU ○ TNC^c, 2.1×10^7 ○ CD34^{+a}, 1.5×10^5 (0.4—3.2)	Tacrolimus + MMF	CBU (CBU engraftment only) ○ Neutrophil, 22 ○ Platelet, 25	98% CBU	No	No acute or chronic GvHD	Yes	20 mo	Alive at last follow-up

Note. CBU = cord blood unit; Cy = cyclophosphamide; DUCBT = double umbilical cord blood transplant; eATG = equine antithymocyte globulin; Flu = fludarabine; Gr = grade; GvHD = graft versus host disease; Haplo = haploidentical graft; HLA = histocompatibility lymphocyte antigen; MMF = mycophenolate mofetil; mo = month; *n* = no. of patients; NR = not reported; OS = overall survival; TBI = total body irradiation; TNC = total nucleated cells; UCB = umbilical cord blood; y = year.

[a] $\times 10^5$/kg of recipient body weight.

[b] $\times 10^4$/kg of recipient body weight.

[c] $\times 10^7$/kg of recipient body weight.

Hence, it is possible that the haplo graft when co-infused with CBU stem cells has some ''altruistic features" and takes the major brunt of the recipient immune response against the graft while creating conducive environment for the survival of the cord graft. Our case supports this hypothesis. Unfortunately, no myeloid versus lymphoid fractionation/chimerism studies were performed. Nevertheless, complete sustained dominance of CBU graft as early as 12 days after HSCT, as seen in our patient, has never been reported before. While it is still in experimental phase, the haplo-cord transplant has the potential to become frontline therapy for patients lacking HLA MRD/MUD. Hence, haplo-cord HSCT seems to be a promising transplant strategy for SAA patients who are refractory to IST and/or lack a well-matched sibling donor. Larger randomized controlled trials are needed to compare this promising HSCT strategy with other established methods of alternative donor HSCT for SAA. Further study should also explore the importance of NK cell alloreactivity as a cause of graft failure before it is labeled as an impediment to engraftment, thereby barring donors who are KIR incompatible. Hence, preclinical and clinical studies to understand the mechanism of transient myeloid engraftment followed by dominant cord chimerism will provide crucial insight into the biology of transplant, thereby leading to further characterization of risk factors and optimization of this transplant strategy. Optimal cell dose, timing of infusion of the grafts, and conditioning regimen need further investigation for their use in haplo-cord HSCT for SAA patients.

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Conflicts of interest

The authors disclose no conflict of interest.

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