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Haploidentical haematopoietic stem cell transplantation for thalassaemia major based on an FBCA conditioning regimen

Qixin Sun,[1,2] Bingyi Wu,[3] Hekui Lan,[4] Fanyi Meng,[5] Xiaoxiao Ma,[4] Xinxin Chen,[4] Zhiwei Huang,[1] Qianqian Yao,[1] Jianhui Xu,[1] Yuxian Huang,[1] Shaojie Wu[1] and Zhigang Zhu[2]

[1]Department of Haematology, Zhujiang Hospital of Southern Medical University, [2]Department of Geriatric Haematology, Guangzhou First People's Hospital, Guangzhou, [3]Department of Haematology, Shunde Hospital of Southern Medical University, Foshan, [4]Department of Paediatrics, Zhujiang Hospital of Southern Medical University, Guangzhou, and [5]Department of Haematology, Kanghua Hospital, Dongguan, China

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Correspondence: Dr Bingyi Wu, Department of Haematology, Shunde Hospital of Southern Medical University, Foshan 52800, China.
E-mail: wubingyi@aliyun.com

Summary

Allogeneic haematopoietic stem cell transplantation (HSCT) is the only available curative therapy for patients with thalassaemia major. With the progress in human leucocyte antigen (HLA) antigen typing technology and supportive care, the outcomes of thalassaemia major have greatly improved in recent years, even in high-risk patients. However, the problem of finding a suitable donor is still a major obstacle to curing these patients. In recent decades, the lack of available HSCT donors has led to the increased use of haploidentical donors (HDs) for HSCT in haematological malignancies. Recently, we explored the effect of HD HSCT to eight children with thalassaemia major based on the FBCA conditioning regimen (fludarabine, busulphan, cyclophosphamide, antithymocyte globulin), which is usually used in leukaemia patients receiving haploidentical HSCT in our centre. So far, all of the transplanted patients have a stable engraftment and are transfusion independent in daily life. This encouraging result has revised our previous conception about haploidentical HCST for thalassaemia major and strongly suggests that HD HSCT is a feasible and safe method for thalassaemia major patients.

Keywords: haematopoietic stem cell transplantation, thalassaemia, haploidentical.

Thalassaemia major is a genetic disease characterized by transfusion-dependent anaemia, iron overload and the resultant damage of internal organs (Angelucci & Pilo, 2016; Choudhry, 2017; Srivastava & Shaji, 2017). Currently, allogeneic haematopoietic stem cell transplantation (HSCT) is the only curative therapy for thalassaemia major. For Pesaro class 1–2 patients, the overall survival (OS) and thalassaemia-free survival (TFS) have been reported as 80–90% (Hussein *et al*, 2013; La Nasa *et al*, 2013; Baronciani *et al*, 2016). However, in reality a human leucocyte antigen (HLA)-matched donor can be found for only 20–30% of thalassaemia major patients (Shenoy & Thompson, 2016). In recent decades, HLA-haploidentical HSCT has been increasingly performed for haematological malignancies (Andreani *et al*, 2017), but the experience is limited for thalassaemia major. To date, only a few small cohort studies have been reported (La Nasa *et al*, 2002; Sodani *et al*, 2010; Anurathapan *et al*, 2016).

In the early 2000s, the Pesaro transplantation group reported the first series of results about thalassaemia major patients transplanted from HLA-mismatched sibling donors (Gaziev *et al*, 2000). Using the classical busulphan/cyclophosphamide-based conditioning regimen, the incidences of OS, event-free survival (EFS), graft failure and acute graft-versus-host disease (aGVHD) were 65%, 21%, 55% and 37%, respectively (Gaziev *et al*, 2000). Subsequently, by adding hydroxycarbamide and azathioprine before transplantation and using a specially selected graft, the incidence of graft rejection and transplant-related mortality (TRM) decreased to 29% and 14%, respectively; and the OS and EFS increased to 90% and 61% (Sodani *et al*, 2010). Pre-transplant immunosuppressant (PTIS) therapy (Gaziev *et al*, 2016; Issaragrisil & Kunacheewa, 2016) and a post-transplant cyclophosphamide strategy(Luznik *et al*, 2008) were applied in a recent thalassaemia major haploidentical HSCT study(Anurathapan *et al*, 2016). Ultimately, 29 of the 31 patients engrafted with 100% donor chimerism, whereas two patients experienced primary graft failure.

Here, we report a new conditioning regimen (FBCA; fludarabine, busulphan, cyclophosphamide, antithymocyte globulin) for thalassaemia major haploidentical HSCT, which used to be performed only in leukaemia patients in our centre and is significantly different from regimens reported before. By analysing the result of this conditioning regimen,

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we hope to provide a new method for thalassaemia major haploidentical donor transplantation.

Patients and methods

Patients and donors

From December 2012 to March 2017, 8 children (4 males and 4 females) with thalassaemia major who received haploidentical HSCT at the Department of Haematology in Zhujiang Hospital were included. The median age of the patients was 5·5 years (range, 3–14). All patients had hepatomegaly and splenomegaly (>2 cm below the costal margin) and one patient had undergone splenectomy. The median ferritin level before transplantation was 2881 μg/l (1562–4219 μg/l).

All donors were HLA-mismatched family members, including fathers, mothers and sisters. The ABO blood type was incompatible between donor and recipient in three cases. The median age of the donors was 31 years (11–40). All donors had received granulocyte colony-stimulating factor 10 μg/kg/day for 5 days. The children's parents signed a consent form in accordance with the Declaration of Helsinki. The therapeutic regimen of this research had been approved by the Ethics Committee of Zhujiang Hospital. The details of the patients and donors are listed in Table I.

Conditioning regimen

The FBCA conditioning regimen consisted of fludarabine (25 mg/m^2/day from days −8 to −3), busulphan (3·2 mg/kg/day from days −7 to −4), cyclophosphamide (60 mg/kg infused over 1 h on days −3 and −2) and rabbit antithymocyte globulin (ATG; 2·5 mg/kg/day infused over 12 h on days −4 to −0).

Graft-versus-host disease prophylaxis

Graft-versus-host disease prophylaxis included ciclosporin (CSA) and short-course methotrexate. CSA was used from day Graft-versus-host disease 1 to maintain a plasma concentration of 200–300 μg/l. Patients were switched to oral CSA whenever they were able to tolerate oral medications. From day +100, the dose was tapered until discontinuation at 1 year. Short-course methotrexate (15 mg/m^2) was intravenously administered at days +1, +3, +5 and +11. If aGVHD was diagnosed, methylprednisolone was intravenously administered at a dose of 2 mg/kg/day. Anti-CD25 monoclonal antibody was administered when methylprednisolone treatment failed.

Monitoring of chimerism

Chimerism was analysed on days +30, +60, +90 and +365 after transplantation. Chimerism of donor/recipient DNA was determined by polymerase chain reaction-based analysis of short tandem repeats (STR). Full donor chimerism was defined as >97·5% donor haematopoietic cells.

Definitions

Neutrophil engraftment day was defined as the day when absolute neutrophil count >0·5 × 10^9/l. Platelet engraftment day was defined as the first of 7 consecutive days when the platelet count was more than 20 × 10^9/l without transfusion. OS was calculated from the day of transplantation until death by any cause. TFS was calculated starting from the day of transplantation until thalassaemia recurrence with transfusion dependence or death. Transfusion independence was defined as a lack of red blood cell transfusion starting 2 months after transplantation.

Statistics

Descriptive statistics were performed on medical records of both patients and donors. OS and TFS were estimated according to the Kaplan–Meier method: $P < 0·05$ defined statistical significance.

Results

Engraftment

The median total nucleated cell dose and CD34$^+$ cell dose in the infused product was 9·7 × 10^8/kg (range, 6·9–26·7 ×

Table I. Patient and donor characteristics.

Patient	Age (patient/donor) (years)	Gender (patient/donor)	ABO blood group (patient/donor)	HLA mismatch locus	Ferritin (μg/l)	Donor relationship to patient	Follow-up (months)
1	5/28	Male/Male	O/O	4/6 (A, B)	1562	Father	7
2	3/30	Female/Male	A/O	3/6 (A, B, DR)	2441	Father	58
3	3/26	Female/Female	O/O	4/6 (A, DR)	4219	Mother	60
4	4/32	Male/Female	B/AB	5/6 (DR)	1766	Mother	38
5	8/11	Female/Female	A/A	4/6 (B, DR)	4189	Sister	41
6	14/36	Female/Female	A/O	4/6 (B, DR)	4205	Mother	34
7	6/32	Male/Male	A/A	6/6 (—)	3322	Father	27
8	12/40	Female/Male	O/O	4/6 (B, DR)	1719	Father	8

Table II. Engraftment and complications of the patients.

Patient	Cell numbers (/kg)		Engraftment (days)		GVHD grade		Virus infection
	Nucleated ($\times 10^8$)	$CD34^+$ ($\times 10^6$)	Neutrophils	Platelets	Acute	Chronic	
1	9·2	12·8	10	11	–	–	EBV
2	26·7	27·2	10	10	II–III	–	EBV
3	10·1	10·4	12	14	–	–	–
4	10·6	9·8	10	13	I–II	–	–
5	8·2	9·3	10	13	I	–	HV
6	9·3	12·3	10	13	I	–	CMV
7	6·9	8·2	13	102	I	–	–
8	12·2	6·9	15	21	III	Local	EBV + CMV

CMV, cytomegalovirus; EBV, Epstein–Barr virus; GVHD, graft-versus-host disease; HV, herpes virus.

10^8/kg) and $10{\cdot}1 \times 10^6$/kg (range, $8{\cdot}2$–$27{\cdot}2 \times 10^6$/kg), respectively. The median time to achieve neutrophil engraftment and platelet recovery was 10 days (range, 10–15 days) and 13 days (range, 10–102 days), respectively. All patients had a stable neutrophil and platelet engraftment after transplantation.

GVHD

Four patients (50%) experienced grade I–II aGVHD. Two patients suffered from grade III–IV (25%) aGVHD, one of which became localised chronic GVHD (cGVHD) of the skin. Acute GVHD was controlled by methylprednisolone. Chronic GVHD was not observed in the other seven patients.

Infection

Virus infection was the second-most common complication after the transplantation. Although Epstein–Barr virus (EBV) DNA replication was detected in three patients, no patient developed post-transplant lymphoproliferative disease (PTLD). Cytomegalovirus (CMV) viraemia was seen in two patients. One patient had been infected by herpes virus (HV) in skin. Veno-occlusive disease (VOD) and haemorrhagic cystitis were not observed in our patients. The detailed results are listed in Table II.

Chimerism and follow-up

Chimerism studies were performed for all patients after transplantation. The data showed that all patients had achieved full donor chimerism (100%) at post-transplantation day +30, and mixed chimerism status was not observed in any patient within the observation period (+365 days). After a median follow-up of 36 months, all patients had survived and had achieved independence from blood transfusion. The OS and TFS rates were both 100% (Fig 1).

Discussion

Allogeneic HSCT is the only available curative therapy for patients with thalassaemia major. However, the lack of a matched family donor always limits HSCT for these patients (Baronciani *et al*, 2016; Chaudhury *et al*, 2017). Although HLA-haploidentical donors, which must be family members, have been thought to solve the problem of HSCT for haematological malignancies(Kanakry *et al*, 2016), the experience for thalassaemia major was still limited.

An early study including 23 patients from the Pesaro transplant group indicated a high rate of graft failure (55%), and severe acute and chronic GVHD (37% and 47%) were major problems of haploidentical-donor HSCT for thalassaemia major(Gaziev *et al*, 2000). To reduce these adverse effects as much as possible, strategies, such as destroying the patient's haematopoietic and immune systems by intensive conditioning regimens (suppressing patient T cell function), selective grafts (high-dose $CD34^+$ stem cells and/or low-dose donor T cells) and post-transplant cyclophosphamide (suppressing patient/donor T cell function), were often applied individually or in combination in thalassaemia major haploidentical HSCT (Luznik *et al*, 2008; Shah *et al*, 2015; Alfraih *et al*, 2016; La Nasa *et al*, 2016; Shenoy & Thompson, 2016).

In 2010, the Pesaro transplant group reported another outcome for thalassaemia major transplantation (Sodani *et al*, 2010). What made that study different was that 22 children were given a T cell depleted allograft ($CD34^+$ cell-positive selection or through $CD3^+/CD19^+$-negative selection) from a haploidentical relative. The cumulative incidences of graft rejection and TRM were 23% and 7%, respectively, which were significantly decreased compared to previous results (Gaziev *et al*, 2000). Finally, the OS and TFS improved to 90% and 61% (Sodani *et al*, 2010). Recently, Anurathapan *et al* (2016) reported the outcomes of 31 thalassaemia major patients given T cell-replete peripheral blood haploidentical HSCT. Their strategy included 2 cycles of PTIS therapy with fludarabine and dexamethasone to all

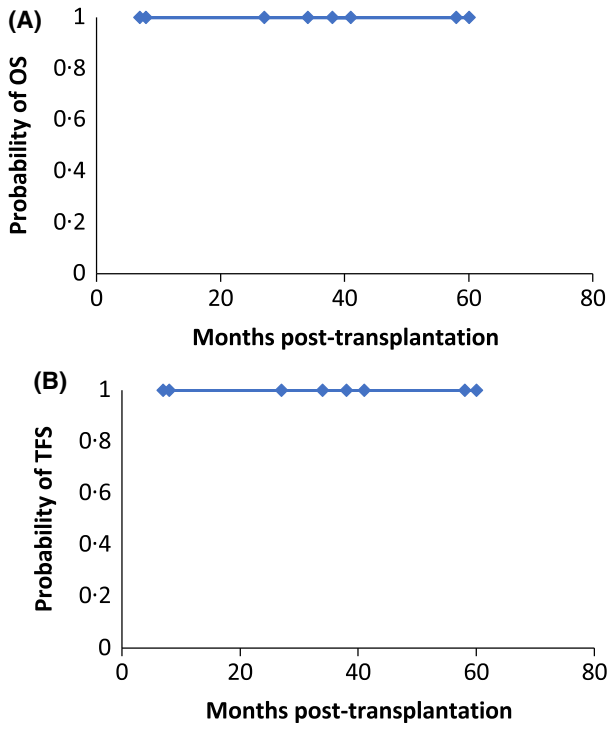

Fig 1. (A) Overall survival (OS) and (B) thalassaemia-free survival (TFS) of thalassaemia major patients treated by haploidentical haematopoietic stem cell transplantation based on an FBCA (fludarabine, busulphan, cyclophosphamide, antithymocyte globulin) conditioning regimen. [Colour figure can be viewed at wileyonlinelibrary.com]

patients and post-transplant cyclophosphamide for GVHD prophylaxis. Twenty-nine patients engrafted with 100% donor chimerism, and two patients experienced primary graft failure. Nine patients (29%) developed aGVHD grade II, and five patients (16%) developed limited cGVHD. Two-year OS and EFS were 95% and 94%, respectively (Anurathapan *et al*, 2016).

Since 2000, we have performed haploidentical HSCT based on the FBCA conditioning regimen for haematological malignancies in our centre. After a median follow-up of 35 months, the 3-year probabilities of OS and disease-free survival for all patients were nearly 63% and 35%, respectively. For eligible patients, the 2-year cumulative incidence of total cGVHD was 24·1%, and that of extensive cGVHD was 5·6% (Lin *et al*, 2015). Considering the low cGVHD and high quality of life of our post-transplant patients, we think a similar effect might be observed in thalassaemia major patients with our transplantation model. Later, from 2012 to 2017, with the consent of their parents, eight thalassaemia major patients received HLA-haploidentical HSCT in our centre. Intensive transfusion and iron-chelating therapy were given by routine treatment before transplantation. All patients were conditioned with the FBCA regimen, and none received PTIS therapy (including azathioprine, hydroxycarbamide and/or dexamethasone) before transplantation. By the end of observation period (+365 days), all the patients had a persistent engraftment (100%) and have achieved blood transfusion independence in daily life. Primary or second graft failure was not observed. Compared to TCD allografts from the Pesaro transplant group, the graft failure in our centre seems to be low. The reason might be the intensity of haematopoietic (busulphan and cyclophosphamide) and immune suppression (fludarabine and ATG) in the conditioning regimen. Additionally, and importantly, no patient suffered TRM in this small cohort. The low TRM might be related to the quick immunological reconstitution post-transplantation in young patients with non-malignant disease (immunological reconstitution data not shown).

Regarding complications, the rate of grade I–IV aGVHD in this group was 75%, whereas that of grade II-IV aGVHD was just 37·5%. Most aGVHD was controlled by steroid therapy. Only one patient developed local cGVHD (12·5%). Our rate of cGVHD was similar to that in T cell-replete haploidentical HSCT plus post-transplantation cyclophosphamide, as reported by Anurathapan *et al* (2016). Due to the small number of patients, it is difficult to comment on whether the incidence of cGVHD in this regimen was significantly low. The incidence of viraemia in our cohort was high. The reason might be related to the use of strong immunosuppressives (fludarabine and ATG). However, so far, no patient has developed PTLD or died from a virus infection.

In conclusion, treatment of thalassaemia major patients with HLA-haploidentical HCST based on the FBCA conditioning regimen is feasible and safe. These results deserve further research and confirmation in larger samples.

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Conflict of interest

The authors declare no conflict of interest.

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Author contributions

BW: designed the clinic study programme, analysed the data and wrote the paper. QS: analysed the data and wrote the paper. SW: performed the research, collected and analysed the data. HL, FM, XM, XC, JX and YH: performed the research and clinic management. ZH, QiY and ZZ: collected and analysed the data.

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