Haematopoietic stem cell transplantation in Nigerian sickle cell anaemia children patients

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ABSTRACT

Background: Sickle cell anaemia (SCA) remains associated with high risks of morbidity and early death. Children with SCA are at high risk for ischaemic stroke and transient ischaemic attacks, secondary to intracranial arteriopathy involving carotid and cerebral arteries. Allogeneic haematopoietic stem cell transplantation (HSCT) is the only curative treatment for SCA. We report our experience with transplantation in a group of patients with the Black African variant of SCA.

Patients and Methods: This study included 31 consecutive SCA patients who underwent bone marrow transplantation from human leukocyte antigen (HLA)-identical sibling donors between 2010 and 2014 following a myeloablative-conditioning regimen.

Results: The median patient age was 10 years (range 2–17 years). Before transplantation, 14 patients had recurrent, painful, vaso-occlusive crisis; ten patients had recurrent painful crisis in association with acute chest syndrome; three patients experienced ischaemic stroke and recurrent vaso-occlusive crisis; two patients experienced ischaemic stroke; one patient exhibited leukocytosis; and one patient exhibited priapism. Of the 31 patients, 28 survived without sickle cell disease, with Lansky/Karnofsky scores of 100. All surviving patients remained free of any SCA-related events after transplantation.

Conclusion: The protocols used for the preparation to the transplant in thalassaemia are very effective also in the other severe haemoglobinopathy as in the sickle cell anaemia with 90% disease free survival. Today, if a SCA patient has a HLA identical family member, the cellular gene therapy through the transplantation of the allogeneic haemopoietic cell should be performed. Tomorrow, hopefully, the autologous genetically corrected stem cell will break down the wall of the immunological incompatibility.

Key words: Bone marrow transplantation, children, sickle cell anaemia

INTRODUCTION

In the sickle cell anaemia (SCA) patient, the synthesis of alfa and beta-globin chains is quantitatively normal, the tetramer being composed of alfa2/beta2 chains. The abnormality is qualitative with the amino acid valine taking the place of amino acid glutamic, in the globin chain sequence of amino acids, with the production of the haemoglobin HbS which has the risk of formation of polymers when the oxygen tension decreases.1 The red blood cells are deformed by the haemoglobin polymerised in fibres and assumed the sickle morphology. Sickle red blood cells form aggregates, occlude small and large vessels, and cause organ infarction. Haematopoietic stem cell transplantation is the only radical cure for SCA with more than 90% successful transplant when the donor is an HLA identical sibling.2-5 We report here our experience in 31 Nigerian children with SCA transplanted from their HLA identical sibling.

PATIENTS AND METHODS

The study included 31 consecutive patients who underwent BMT from an HLA-identical sibling donor between 2010 and 2014. The Institutional Review Board approved the treatment protocol, and all parents or patients provided written informed consent, in accordance with the Declaration of Helsinki.
All patients received cyclosporine A, low-dose methylprednisolone, and a short course of methotrexate as GVHD prophylaxis. All patients received bone marrow from HLA-identical sibling donors 36 h after the final dose of cyclophosphamide. All donors with sickle cell trait received hyper hydration and blood transfusion before the multiple marrow aspirations. In surgery, pre-operative transfusions are given to prevent peri-operative complications during bone marrow aspirations in sickle cell trait donors. Diagnosis and degree of acute and chronic GVHD was assessed according to consensus criteria.6,7

As anticonvulsant prophylaxis all patients received valproic acid (Depakin®, Sanofi-Aventis, Milano, Italy) at a dose of 30 mg/kg/day in three divided doses starting at 24 h before the first Bu administration and continuing until the patients were off cyclosporine. All patients were administered prophylactic broad-spectrum antibiotics and antifungal drugs until the neutrophil level exceeded 1.0 × 10⁹/L. Patients also received acyclovir as herpes virus prophylaxis and trimethoprim/sulfamethoxazole as *Pneumocystis jiroveci* prophylaxis. From the beginning of transplantation preparation until at least 100 days post-transplantation, all patients were monitored weekly for the presence of Epstein-Barr virus, cytomegalovirus (CMV), adeno virus, and BK virus in the blood and/or urine using sensitive reverse transcriptase PCR.

All patients had the first chimerism analysis performed on bone marrow samples 20 days after transplantation. The percentage of donor/recipient DNA was determined by PCR-based analysis of short tandem repeats (Profiler Plus Applera). At 60, 90, 180, and 365 days after transplantation, we determined myeloid and lymphoid chimerism.

The study endpoints were overall survival (OS), sickle cell free (SCF) survival, graft-versus-host disease (GVHD), graft rejection, and transplant-related mortality (TRM). SCF survival was defined as survival without graft rejection or death. OS was defined as time from transplant to death, irrespective of the cause. The day of neutrophil engraftment was defined as the first of 3 consecutive days during which the absolute neutrophil count was 0.5 × 10⁹/L or higher. Platelet engraftment was defined as the first of 7 consecutive days during which platelet counts exceeded 20 × 10⁹/L in the absence of transfusion.

### Statistical analysis

The probabilities of survival, SCA-free survival, rejection, and mortality were calculated using the Kaplan-Meier estimator.8

### RESULTS

Patient pre-transplant demographics, disease and transplant characteristics are described in Table 1. Before transplantation, 14 patients had recurrent, painful, vaso-occlusive crisis; 10 patients had recurrent painful crisis in association with acute chest syndrome; three patients experienced ischaemic stroke and recurrent vaso-occlusive crisis; two patients experienced ischaemic stroke; one patient exhibited leukocytosis; and one patient exhibited priapism.

All patients had sustained engraftment. The median time to ANC ≥500 × 10⁹/L was 16 days (range, 11-23 days) and median time to a platelet count ≥20 000 × 10⁹/L was 16 days (range, 11-22 days). At 2 months after transplantation three patients had donor chimerism between 95% and 98%, and all the remaining patients had full donor chimerism. At the last control all patients experienced sustained engraftment with 100% donor chimerism. Post-transplantation outcome of SCA patients is reported in Table 2.

Seven patients developed grade 2 acute GVHD and five patients developed grade 3-4 GVHD. All patients responded promptly to the steroid treatment administered to control acute GVHD. At present, all Black-African variant patients except one are off immunosuppressive medication. Chronic GVHD was

| Variables | Patients, n = 31 |
|-----------|------------------|
| Patient sex: M/F | 17/14 |
| Conditions before transplant, n | 2 |
| Stroke | 14 |
| Recurrent vaso-occlusive crisis | 10 |
| Recurrent acute chest syndrome and recurrent vaso–occlusive crisis | 3 |
| Vaso–occlusive crisis and stroke | 1 |
| Recurrent hand foot syndrome and leukocytosis | 1 |
| Recurrent priapism | 1 |
| Median donor age, years | 11 (1-25) |
| Median Hb, gr/dl | 7.8 (5.7-10.7) |
| Median Hb F, % | 12.6 (1.9-28) |
| Median Hb S, % | 81.6 (61-94.2) |
| Median bilirubin, mg/dl | 2.3 (0.5-5.5) |
| Donor / patient CMV serology: Both positive | 30 |
| Both negative | 1 |
| Median nucleated cell dose, × 10⁹/kg | 3.9 (1.5-10) |
| Median CD34 + cell dose, × 10⁹/kg | 5.9 (1.2-11.3) |
observed in four patients. One patient developed bronchiolitis obliterans, and one patient had severe chronic GVHD with intestinal and hepatic involvement until death as a result of multi-organ failure at day +190 post-transplantation. Cumulative incidence of grade 3-4 acute GVHD was 16%. Cumulative incidence of persistent severe chronic GVHD was 13%.

Six patients had cyclosporine A-related neurotoxicity with seizures. All patients received valproic acid (Depakine; Sanofi-Aventis) at a dose of 30 mg/kg/day in three divided doses starting at 24 hours before the first busulfan administration. Many risk factors for the development of CSA-related neurotoxicity have been investigated in our patients, including arterial hypertension, fluid overload, hypercholesterolemia, hypomagnesaemia and pre-existing brain disease. In the screening examinations of these patients the brain magnetic resonance imaging (MRI) showed gliosis in 11/31 stroke free Black-African SCA patients. The brain MRI finding usually associated to CSA neurotoxicity was posterior reversible leukoencephalopathy syndrome (PRES), typically distributed in the posterior regions of the white matter of the brain. In general the prognosis of CSA neurotoxicity has been good and posterior leukoencephalopathy usually resolved completely with dose reduction or drug withdrawal. As alternative GVHD prophylaxis, we opted for tacrolimus. This calcineurin inhibitor, although similar to CSA in mechanism and metabolism, did not produce neurological side effects in these patients.

All patients except one had positive serology for CMV before transplantation. Asymptomatic CMV reactivation occurred in 29 of 31 patients. All patients were provided pre-emptive antiviral therapy and none developed CMV disease. One patient had pneumonia due to aspergillosis, which resolved with antifungal therapy and surgical removal of the infected lung cavity; one patient experienced aspergillosis in the upper airway, which resolved with antifungal therapy. Seven patients developed BK virus-related grade 2 haemorrhagic cystitis, which resolved with cidofovir therapy.

### Table 2: Post-stem cell transplantation outcome of SCA patients

| Days after Transplantation | Donor Engraftment | Acute GVHD | Clinical Conditions | Haemoglobin |
|----------------------------|-------------------|------------|---------------------|-------------|
|                            | %                 | % HbS      | % HbA1              | % HbF       |
| 1468                       | 100               | 0          | Alive and cured     | 30          |
| 1019                       | 100               | 0          | Alive and cured     | 41          |
| 1019                       | 100               | 2          | Alive and cured     | 37          |
| 984                        | 100               | 0          | Alive and cured     | 0           |
| 922                        | 100               | 0          | Alive and cured     | 36          |
| 880                        | 100               | 0          | Alive and cured     | 0           |
| 872                        | 100               | 3          | Alive and cured     | 35          |
| 802                        | 100               | 4          | Died                | 0           |
| 802                        | 100               | 2          | Alive and cured     | 0           |
| 774                        | 100               | 2          | Alive and cured     | 0           |
| 705                        | 100               | 2          | Alive and cured     | 41          |
| 662                        | 100               | 3          | Died                | 37          |
| 656                        | 100               | 2          | Alive and cured     | 0           |
| 648                        | 100               | 0          | Alive and cured     | 40          |
| 642                        | 100               | 0          | Alive and cured     | 0           |
| 620                        | 100               | 0          | Alive and cured     | 28          |
| 572                        | 100               | 4          | Died                | 0           |
| 564                        | 100               | 0          | Alive and cured     | 35.8        |
| 523                        | 100               | 0          | Alive and cured     | 0           |
| 515                        | 100               | 0          | Alive and cured     | 0           |
| 481                        | 100               | 0          | Alive and cured     | 37          |
| 473                        | 100               | 2          | Alive and cured     | 42.1        |
| 445                        | 100               | 0          | Alive and cured     | 0           |
| 431                        | 100               | 3          | Alive and cured     | 0           |
| 417                        | 100               | 0          | Alive and cured     | 41.3        |
| 422                        | 100               | 0          | Alive and cured     | 39.4        |
| 390                        | 100               | 0          | Alive and cured     | 47.3        |
| 323                        | 100               | 0          | Alive and cured     | 39.3        |
| 77                         | 100               | 0          | Alive and cured     | 35.8        |
| 74                         | 100               | 0          | Alive and cured     | 38          |
| 53                         | 100               | 0          | Alive and cured     | 35          |

HbS: Haemoglobin S; HbA1: Haemoglobin A1; HbA2: Haemoglobin A2; HbF: Haemoglobin F; na: Not available
Twenty-eight out of 31 SCA patients survived without sickle cell disease, with Lansky/Karnofsky scores of 100. One patient died at 77 days post-transplantation from complications of acute GVHD. One patient died from multi-organ failure at 190 days post-transplantation. He had no steroid responsive grade 4 acute GVHD of gut and developed sepsis, which led to multiorgan failure and death. One patient died from complications of bronchiolitis obliterans at 445 days post-transplantation. After transplantation, no patients experienced complications typical of SCA, such as pain, stroke, or acute chest syndrome.

The cumulative probabilities of survival, SCA-free survival, and transplant-related mortality were 90%, 90%, and 10%, respectively [Figure 1].

DISCUSSION

Numerous studies on haematopoietic stem cell transplantation in SCA report a disease-free survival ranging between 80 and 90%;9-14 yet, there is not a large acceptance of transplantation in SCA report a disease-free survival ranging between 80 and 90%;9-14 yet, there is not a large acceptance of transplantation in SCA. Consequently, there is a need to categorize the risk of transplantation for these patients, as was done for thalassaemic patients. The timing of transplantation is more problematic in patients with SCA compared to those with thalassaemia, where there is great phenotypic similarity in patients of the same age. Clinically, there are large polymorphisms in SCA.15,16 Allogeneic cellular gene therapy involves replacement of the defective haemopoietic system with an allogeneic haemopoietic stem cell, which acts as a cellular vector for the normal gene regulating globin synthesis. As in thalassaemia, this procedure is the only radical cure for patients with SCA.

We think allogeneic cellular gene therapy, through haemopoietic stem cell transplantation, should be offered to all SCA patients, when an HLA identical family member is available. Allogeneic cellular gene therapy after HSC transplantation should be considered early in the course of this disease that inevitably becomes more severe with time.

Here we report our experience with transplantation in Black-African variant SCA children patients (all from Nigeria). All patients received transplants from an HLA-identical sibling. The results summarised in Figure 1 show an OS of 90% and an event-free survival of > 80%.

Children with Black-African variant SCA are prone to invasive infections caused by *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Plasmodium falciparum* (in malarious areas). Malaria is more endemic in Black-African areas and therefore malaria is more common in black SCA patients. In Africa, malaria contributes substantially to the early mortality of patients with SCA. The progressive splenic hypo-function due to repeated infarcts leads to the breakdown of acquired immunity. *Plasmodium falciparum*, therefore, is capable of infecting HbSS cells in vivo in the absence of acquired immunity. So, the HbS advantage appears to be lost when acquired immunity is defective. For these reasons we preferred in this population fludarabine-based preparative protocols, well tolerated, with less immunosuppression and minimal toxicity respect ATG (manuscript in press).

In conclusion, HSCT should be considered for each patient diagnosed with SCA and with an HLA-identical donor as soon as possible, before they develop disease and treatment-related irreversible organ damage.

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