Stem Cell Transplantation for Severe Aplastic Anaemia in Children: A Single Institute Experience

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Abstract

Fifteen children diagnosed of severe aplastic anaemia were treated with allogeneic haematopoietic stem cell transplantation (HSCT). The patients presented at the median age of 8.5 years, and 7 of 15 patients received HSCT within 2 months after diagnosis. The other patients had received immunosuppressive treatment but failed to response, they were then subsequently transplanted at 8-208 months after diagnosis. Ten patients had Human Leukocyte Antigen (HLA) identical sibling HSCT, whereas the other 5 patients received 1-3 antigen mismatched family donors (n=4) or unrelated donor transplant (n=1). Except two patients, all the other patients had successful engraftment of donor cells at median of 19 days. Those with successful engraftment did not have significant complications, and 5/13 patients developed graft versus host disease which responded to immunosuppressive treatment. Two patients without engraftment subsequently died of infection. The overall survival for the whole group was 86%, and it was 100% for HLA identical or mismatched sibling transplant. At a median follow up of 7 years, the survivors were all in good health without late morbidity. In conclusion, HLA identical sibling HSCT achieved a high chance of cure and is the treatment of choice for children with severe aplastic anaemia.

Key words

Aplastic anaemia; Immunosuppressive therapy; Stem cell transplantation

Introduction

Severe aplastic anaemia (SAA) is a rare disease in children with an incidence of 2-3 per million paediatric population. Most of the cases are idiopathic without underlying causes, but the pathogenesis is thought to be immune mediated. Before the era of potent immunosuppressive treatment, SAA carried a high morbidity and mortality rate. With the introduction of anti-lymphocyte globulin or anti-thymocyte globulin and cyclosporine, the survival is now improved to 70% at 5 years after diagnosis. However some patients only have partial response or relapse after initial response. Some patients are dependent on long term immunosuppressive treatment. The long term disease free survival after immunosuppressive treatment is about 50-60%. Allogeneic bone marrow transplantation from a Human Leukocyte Antigen (HLA) compatible sibling is the preferred treatment option because it is associated with a high cure rate. We report the result of allogeneic haematopoietic stem cell transplantation (HSCT) for SAA in a single institute over 15 years.

Patients and Method

Patients were diagnosed acquired SAA if they fulfilled the following criteria: (1) severe anaemia that was transfusion dependent, (2) absolute neutrophil counts <0.5 x 10^9/L, (3) platelet count <20 x 10^9/L, (4) a hypoplastic bone marrow on biopsy with cellularity <5%. There should...
be no abnormal cytogenetic findings in the bone marrow karyotyping. Congenital aplastic anaemia syndromes were not included in this series. Newly diagnosed SAA would be HLA typed with the family members soon after diagnosis. Patients would be treated with triple immunosuppressive therapy (IST); anti-thymocyte globulin and cyclosporine and steroid; when no HLA identical family donor available. HSCT would be offered when a HLA identical sibling was available. Blood transfusion was minimised while waiting for HSCT. When patients failed to respond to immunosuppressive treatment, i.e. at least 2 courses of IST at 3 months apart, they would be offered unrelated donor HSCT or mismatched family donor transplant. Informed consent was obtained from parents or patients before HSCT.

A central venous catheter was inserted before the start of preparative chemotherapy for HSCT. The patients were all nursed in laminar air-flow isolation rooms in the Bone Marrow Transplant (BMT) unit. The preparative regimen was standardised for HLA identical siblings. Cyclophosphamide was given at 50 mg/kg per day iv over one hour for 4 consecutive days. Hyperhydration and mesna prophylaxis would be administered to prevent haemorrhagic cystitis. Anti-thymocyte globulin was given at 30 mg/kg per day iv over 6 hours for 3 consecutive days. Haematopoietic stem cells were infused on day of transplantation, and must be at least 36 hours from last dose of cyclophosphamide. The choice of stem cell was initially bone marrow, and then was later extended to peripheral blood stem cells or umbilical cord blood.

Mismatched donor transplant was prepared by a more immunosuppressive preparative regimen. Anti-thymocyte globulin was given at the same dose as sibling transplant, cyclophosphamide was given as 60 mg/kg iv for 2 consecutive days. In addition, fludarabine 30 mg/m² iv daily for 5 consecutive days. The patient received unrelated donor also received total body irradiation 6 Gy over 2 days. Graft versus host disease prophylaxis was cyclosporine 1.5 mg/kg iv Q12H from one day before transplantation and changed to oral dose when tolerating feeding. Methotrexate at 10-15 mg/m² iv for 4 doses in the first 2 weeks.

The engraftment of neutrophil was defined as the first day of 3 consecutive days with neutrophil >0.5 x 10⁹/L. Platelet engraftment was defined as the first day of platelet-independent for at least 7 days and platelet >20 x 10⁹/L. The cell origin of haematological cells was checked by fluorescent in-situ hybridization for sex chromosomes if the donor and recipient were sex-mismatched. Sex matched pairs were checked by short tandem repeats using polymerase chain reaction. The survival was estimated by Kaplan Meier curve. Death or rejection of donor stem cells was defined as event.

Results

From 1991 to 2004, a total of 15 acquired SAA patients received haematopoietic cell transplantation at BMT Unit of Prince of Wales Hospital. There were 12 females and 3 males. The median age at presentation of aplastic anaemia was 8.5 years, ranged from 1.3 year to 12.8 years. The time of diagnosis to transplantation was much shorter in HLA identical sibling, 7 of 10 patients received transplantation in less than 2 months. Three patients were treated with immunosuppressive treatment but failed. These 3 patients were transplanted at 8 months, 8 months and 208 months after diagnosis. Whereas mismatched transplant cases were transplanted after failure of IST and at a much longer period of observation, from 15 months to 44 months.

Ten patients received HLA identical sibling transplantation, while 2 received one antigen mismatched related donor transplantation (one from brother, one from mother). One patient received 3-antigen mismatched umbilical cord blood transplantation, while another received haploidentical transplantation from father. One patient received HLA matched unrelated donor transplantation. The median age at time of transplantation was 11 years, ranged from 1.3 years to 22.7 years. The stem cell source was bone marrow in 4 cases, all were from HLA identical sibling transplantation. Ten patients received peripheral blood stem cell transplantation: 6 HLA identical siblings, 2 one-antigen mismatched relatives, 1 haploidentical mother, 1 unrelated donor. The stem cells of haploidentical transplantation were specially prepared with CD34 cells selection, and the T-cells were depleted significantly to prevent graft versus host disease. One patient received mismatched related umbilical cord blood transplantation.

Engraftment and Survival

Thirteen of 15 patients achieved donor cell engraftment. Two patients did not have any sign of donor cell engraftment, one from unrelated donor and one from haploidentical father. The median day of neutrophil engraftment was 19 days (14-24), and the day of platelet engraftment was 21 days
Acute graft versus host disease occurred in 5 patients: 3 with grade II, 1 with grade III and another one with grade IV. They were treated with methylprednisolone and all showed good response. Four patients developed chronic graft versus host disease and all were limited to skin. The graft versus host disease subsequently resolved after cyclosporin and prednisolone treatment.

With a median follow up of 7 years, from 1 year to 14 years, 13 patients survived in disease-free. Two patients died from infection after failure of engraftment of donor cells. One died of HHV 7 pneumonia 10 days after second transplant, and another died of klebsiella septicaemia at 4 months after transplant. The probability of 5 years disease free survival for the whole group was 86%. For HLA identical or mismatched sibling HSCT, the 5 year disease free survival was 100%. Only 3 of 5 patients received transplants from sources other than HLA identical sibling survived in remission. There was no long term complications detected in the survivors, and they had normal growth and puberty.

Discussion

SAA is an uncommon disease in children but it carries a high mortality if the patient does not respond to immunosuppressive treatment. The commonly used triple immunosuppressive therapy has been reported for more than 10 years, and still remains the most effective regimen in SAA. The response rate was reported to be 40-70% at 4 months after start of treatment. With long term follow up of up to 10 years, the relapse rate was 38%, and 26% of patients were still cyclosporine dependent. Clonal or malignant disease occurred up to 25% in these patients, some developed acute myeloid leukaemia or myelodysplastic syndrome. The long term survival was thus only 54-58% at 11 years. Immunosuppressive therapy may achieve response in some patients but the abnormal clone causing the disease may still persists with clonal transformation later. While HSCT appears to provide long term survival of cure, there was no report of clonal transformation or second malignancy in those patients not receiving irradiation as part of the preparative regimen.

The preparative regimen of HSCT in SAA aims at providing strong immune suppression to allow the engraftment of donor cells. The purpose of cyclophosphamide and anti-thymocyte globulin is for immune suppression. Myeloablative chemotherapy such as busulphan or total body irradiation is not required. Cyclophosphamide alone is not potent enough to prevent graft rejection which may be as high as 30-60%. Thus the combined use of cyclophosphamide and anti-thymocyte globulin is now a standard regimen in most transplant centres. Storb et al reported the results of 4 large BMT centres using this regimen in US from 1988-1999. Ninety-four patients received standard preparative regimen, of these 38 had received prior immunosuppressive therapy before BMT. Graft rejection only occurred in 4%, grade II-IV acute graft versus host disease happened in 29% patients. At a median follow up of 6 years, 88% patients survived in remission. In our series, the 10 HLA identical sibling transplantations all had successful engraftment and remained in long term survival. Late complication was not observed and the patients had normal growth.

The French investigators reported the experience of transplantation in Paris. One hundred patients received cyclophosphamide as preparative regimen, and in addition thoracoabdominal irradiation was given at 6 Gy. The 10 year survival was 65% but 9 patients developed solid cancers at 1.2-17 years after BMT, usually within the radiotherapy field. The centre subsequently changed the preparative regimen to combination of cyclophosphamide and anti-thymocyte globulin and reported the survival of 90% without second malignancy. The use of irradiation in HLA identical sibling setting is now abandoned by all the centres. However in the setting of mismatched transplantation or unrelated donor transplantation, total body irradiation or total lymphoid irradiation is included in the preparative regimen as this provides more potent immunosuppressive treatment. Alternatively a more immunosuppressive regimen including fludarabine has also been reported for mismatched donor transplant. We included total body irradiation in our only case of unrelated donor transplant, but unfortunately there was no successful engraftment. Haploidentical transplantation has recently been introduced for patients who cannot get a matched related and unrelated donor. A high stem cell dose may overcome the HLA disparity barrier and long term survivors have been reported. However SAA patients are well known to have high graft rejection rate due to repeated blood transfusion, the use of unrelated donor and haploidentical parent transplant should only be considered as the last resort.

In conclusion, HLA identical sibling transplantation achieved a high cure rate without significant long term complication. This is the recommended treatment for newly diagnosed SAA. The mismatched donor or unrelated donor...
transplantation should be reserved for those who failed immunosuppressive treatment.

References