original research report

Outcome of allogeneic stem cell transplantation with a conditioning regimen of busulfan, cyclophosphamide and low-dose etoposide for children with myelodysplastic syndrome

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BACKGROUND AND OBJECTIVES: Allogeneic stem cell transplantation (SCT) offers the best chance of cure and long-term survival for children with myelodysplastic syndromes (MDS).

DESIGN AND SETTING: Retrospective analysis of pediatric patients with primary MDS treated with allogeneic SCT at a single institution treated between January 1993 and December 2008.

PATIENTS AND METHODS: Of 16 consecutive children who received allogeneic SCT for treatment of MDS in our center, 14 patients met the criteria of MDS according WHO I and II criteria. The median age was 4.8 years (range, 1-14 years) and 64% were male. The median time from diagnosis to transplant was 6 months. MDS stage was refractory cytopenia (RC) in 9, refractory anemia with excess blasts (RAEB) in 5. Monosomy 7 was present in 35% of the patients. The majority of patients (11/14) were conditioned with a busulfan-based myeloablative (MA) regimen with addition of low-dose of etoposide (30 mg/kg). All but one received a bone marrow graft.

RESULTS: Nine patients achieved complete remission (CR), and seven remain alive. At a median follow-up of 3 years (range, 2-14 years) the OS and EFS was 57% (95%CI, 0.28-0.78). Cumulative EFS at 10 years was 43% (95% CI: 0.14–0.70). Relapse-related mortality was 21.4%; nonrelapse mortality (NRM) was 28.57%. All the survivors had etoposide in their conditioning regimen. Patients younger than 10 years had better survival (P=.001).

CONCLUSION: Children with MDS achieve encouraging OS and EFS following allogeneic SCT. A busulfan-based regimen with a lower dose of etoposide is an effective and less toxic regimen. The outcomes are best in younger patients.

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal bone marrow stem cell disorders, characterized by ineffective erythropoiesis, a variable degree of cytopenia, and an increased risk for developing acute myelogenous leukemia (AML). MDS is rare in children (incidence <3-4/1 000 000 children.[1] Those affected almost always have advanced disease (RAEB-I and RAEB-II) and progress to leukemia more often than adults.[2,3]The majority of children also have associated chromosomal abnormalities, the most frequent of which is monosomy 7.[4]

The MDS classification that includes some of the FAB subtypes (juvenile myelomonocytic leukemia [JMML], the monosomy 7 syndrome and others) has not been universally accepted and today there is a consensus that these disorders are distinct from MDS.[5] An international consensus has been recently achieved on the classification of MDS in childhood. This classification includes refractory cytopenia (RC) and refractory anemia with excess blasts (RAEB).[6, 7] Therapy-related MDS are generally considered separately, given the different etiology, clinical characteristics, and poorer prognosis.[8,9]

Table 1. Patient clinical and transplantation characteristics.

Characteristic	Number of patients (unless noted otherwise)
Median age, year (range)	4.8(1-14)
Gender (male/female)	9/5
Time from diagnosis to transplant, months (range)	6 (3-22)
MDS Subtype according WHO-II	9
Refractory cytopenia of childhood REAB	5
Cytogenetics	
Normal	6
Monosmy 7	5
Others	3
Graft	
Bone marrow	13
Cord blood	1
CD34/kg	6.28×10^6 (1.2-13)
Conditioning	
BUS/CYC/VP16	11
BUS/CYC	2
BUS/CYC/ATG	1
GVHD prophylaxis	
CSA	2
CSA/MTX	11
CSA/prednisone	2

RAEB: refractory anemia with excess blasts; UCOB: unrelated cord blood; BUS: busulphan; CYC: cyclophosphamide; ATG: anti-thymocyte globulin; GVHD: graft-versus-host disease; CSA: cyclosporine; MTX: methotrexate.

The intent of treatment in children with MDS is curative whereas in adults palliation is often the most feasible approach. Even though spontaneous remissions of MDS with monosomy 7 have been reported, these are rare and do not preclude initiation of therapy with a curative intent.[10] Intensive chemotherapy, similar to treatment in patients with newly diagnosed AML, can induce remissions in 15% to 60% of patients, but these remissions are not durable, resulting in high relapse rates and overall survival (OS) rates of <30%.[11-13]Allogeneic SCT, using bone marrow (BM) from matched related donors (MRD) offers the best chance for cure with long-term survival.[14-16] Alternative donors such as matched unrelated donors (MUD), unrelated cord blood (UCB), or partially matched related donors are also available options in the absence of a full-matched donor.[17-19]

The paucity of reports on the outcome of SCT for MDS in the pediatric literature can be explained by several factors, including the rarity of MDS in children, the lack of a widely accepted classification, the reporting of pediatric populations within adult studies, and reporting MDS with other myeloproliferative disorders such as JMML, CMML, or therapy related AML (t-AML). This study presents the outcome of allogeneic SCT in children with MDS who were retrospectively classified according to the new WHOII classification for childhood MDS.[7]

PATIENTS AND METHODS

Between January 1993 and December 2008, 16 children underwent allo-SCT for treatment of MDS in our center. The diagnosis of MDS was based on an evaluation of the bone marrow aspirate, bone marrow biopsy, peripheral blood smear and cytogenetic studies. Fourteen patients met the criteria of MDS according WHO I and II criteria. Patient characteristics at transplant are summarized in **Table 1**. The median age at SCT was 4.8 years and disease duration pre-SCT ranged from 2 to 12 months with a median of 6 months. The majority of patients presented with symptoms relating to neutropenia, thrombocytopenia, and anemia. Cytogenetic studies were available on all patients, and five had monosomy.[7]

Treatment prior to transplant consisted of red blood cell transfusions in 4 patients, platelet transfusions in 6 patients and granulocyte-colony stimulating factor (G-CSF) in 3 patients with RC. Three patients with RAEB received AML-type chemotherapy. The three patients achieved complete remission (CR-1) prior to SCT.

The source of the stem cells was bone marrow from an HLA-matched sibling or parent in 13 patients and UCB in one patient. Harvested marrows were not manipulated, and the median CD34 dose in matched-related was 6.3×10^6 per kg recipient body weight (range $2.3\text{-}13\times 10^6$).

All patients were conditioned with an myeloablative regimen. This regimen consisted of intravenous (IV) busulfan (BU) at 4 mg/kg daily in four divided doses for 4 days (total dose 16 mg/kg), IV cyclophosphamide (CY) at 50 mg/kg daily for 4 days (total dose 200 mg/kg) and IV etoposide (VP-16) at 300 mg/m^2 daily for 3 days (total dose 900 mg/m^2). Eleven patients received BU/CY/VP16; three patients received BU/CY only. The choice of conditioning regimen was based on current practices within the institution at that time. GVHD prophylaxis was with cyclosporine A (CSA) and methotrexate in 11 patients, CSA and methylprednisone in one patient and CSA only in two patients.

All patients were housed in HEPA-filtered rooms

and were isolated until engraftment. Intravenous immunoglobulin (IVIG) was administered every 2 weeks until day +90. All patients received aciclovir and no prophylactic antifungal therapy was given. All blood products were leukocyte-filtered and irradiated.

Engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count >0.5/L×10^9/L. Platelet recovery was defined as a platelet count >20×10^9/L for 7 days without any transfusion. Relapse was identified by recurrence of a prior cytogenetic abnormality or by reappearance of morphologic evidence of disease. Graft failure was defined as a lack of donor cell engraftment by 28 days post-SCT and graft rejection as the loss of donor cells following engraftment with or without return to host hematopoiesis. Chimerism was assessed by FISH for sex mismatched transplants, or by variable tandem repeats (VTNR) for the same sex, while from January 2004 onwards, it was assessed by short tandem repeats (STR).

OS and event-free survival (EFS) were obtained by the Kaplan-Meier method. The significance limit for *P* values was set at .05 in all tests. The χ^2 test was used to compare frequencies. Death from any cause, relapse, graft failure and secondary malignancy were considered events for EFS. Outcomes were analyzed from the time of the patient's stem cell infusion (day 0) until the first subsequent event or, for event-free patients, the time of the last follow-up.

RESULTS

Twelve (85.7%) patients engrafted; the median time to engraftment was 18 days (range, 11-32 days) for neutrophils and 28 days (range, 17-46 days) for platelets. Chimerism studies at the last contact were available for 6 patients and all had complete donor chimerism. Five patients (35.71%) developed grade II acute GVHD which responded to treatment. Chronic GVHD of the liver and skin was seen in two patients, both responded to treatment with steroids and mycophenolate. No veno-occlusive disease (VOD), hemorrhagic cystitis or skin toxicity with etopside were observed. CMV infection developed in three patients and resolved with ganciclovir therapy.

Three patients relapsed after complete engraftment at a median of 99 days post-transplant (range, 55-110 days) two patients had RC and monosomy 7 and one had RAEB with complex cytogenetics. These patients died shortly after relapse secondary to disease progression. Four patients (28.57%) died at a median of 6 months post-transplant (range, 2-41 months). Causes of death were graft failure in two patients, and septic shock secondary to immune-suppressive therapy for chronic GVHD in two patients. Nine patients achieved CR and seven patients remain alive. At a median follow-up of 3 years (range 2-14 years) the OS and EFS was 57% (95%CI, 0.28-0.78) (**Figures 1a, b**). Cumulative EFS at 10 years was 43% (95% CI, 0.14-0.70). Univariate analysis showed that patients younger than 5 years had a better survival (*P*=.001). However, neither the MDS subtype (*P*=.530) nor the presence of monosomy 7 (*P*=.592) had an impact on the EFS. No

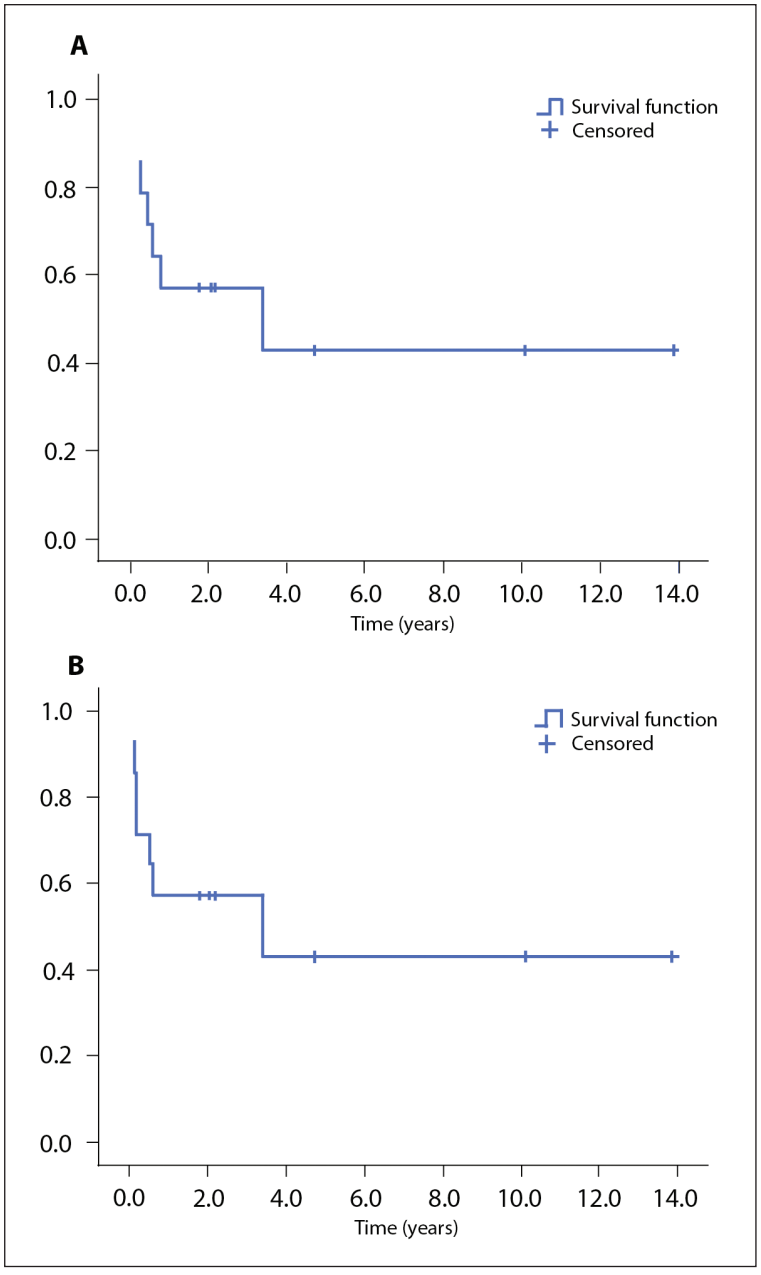

Figure 1. Kaplan-Meier plot for (A) overall survival and (B) event-free survival, 3-year OS and EFS; 0.571±0.132 (95%CI: 0.28-0.7857%).

cases of secondary malignancy were noted at median follow-up of 8 years (range, 2-14 years).

DISCUSSION

Allogeneic SCT currently offers the best chance of cure and long-term survival for children with MDS.[14-16] In this article we report the outcome of pediatric patients with MDS treated with allogeneic SCT at a single institution. To study a more homogeneous cohort of patients, and in compliance with the recent WHO classification, we excluded patients with JMML and secondary MDS from this analysis.

All patients received myeloablative conditioning regimens, which for most patients was BU/CY/VP16. Bu/Cy regimen alone or in combination with other agents provided an effective alternative to Cy/TBI in patients with AML and myelodyplasia.[20,21] TBI-based regimens tend to be associated with an increased incidence of NRM, acute GVHD, and growth failure.[15] The safety profile of etoposide as part of the conditioning regimen has been widely explored in adult studies.[20] In children, however, few studies have addressed the use of high-dose etoposide (40-60 mg/kg) in combination with other agents in the preparative regimens with lower dose of BU/CY. Although these studies showed that etoposide was fairly well tolerated, the investigators failed to show a clear advantage to using etoposide in AML patients.[21,22] In this series, standard doses of Bu/Cy were used in addition to low dose of etoposide (30 mg/kg). The addition of etoposide was not associated with any unusual toxicity or delayed engraftment and there was no increased incidence of VOD or GVHD, and quite notable was the absence of skin toxicity. While we can conclude that the combination of Bu/Cy with a lower dose of etoposide is a safe and effective myeloablative regimen for MDS, the small sample size of our study and the inability to conduct comparative analysis with published data due to heterogeneity of conditioning regimens used in these studies precludes validation of this observation.

Secondary malignancies, particularly acute leukemia, are potential late effects of etoposide. TBI-based regimens also increase the risk of secondary malignancy, including solid tumors. This issue was not addressed in many of the previously published series, but it should be considered when choosing the preparative regimen. In our study, with median follow up of 6 years, no secondary malignancy was noted, although longer term follow-up is probably necessary, since the median time to development of epipodophyllotoxins related t-AML is short. It is likely that at least that risk may have been avoided.

The overall rate of EFS for MDS patients has ranged between 15% and 58% with relapse rates of 14% to 26%, and NRM of 40%. The limitation of these studies lies in the inclusion of patients with JMML and t-AML.[11-15] With an EFS of 57% and non-relapse mortality (NRM) of 28.6%, our results compare favorably with these reported studies. Recently Munoz et al[23] reported the experience of the Spanish Working Party for Blood and Marrow Transplantation in Children (GETMON) with the use of allogeneic SCT from matched related donors, unrelated donors and cord blood in 24 patients with MDS applying the new WHO classification. The estimated EFS for all patients at 5 years was 38%. The estimated EFS at 5 years for patients in the MRD group was 48% versus 25% for the patients in the MUD/UCB groups. The 5-year cumulative incidence of transplant-related mortality (TRM) in the MRD group was 33% versus 50% in the MUD/UCB groups (P=.05). Our study showed a similar EFS, and less incidence of NRM and GVHD. This could be explained by the conditioning regimen used in this study. Contrary to what was reported by Parikh et al and Munoz et al[19,23]our study showed that age may have an impact on EFS with older patients doing better. Monosomy 7 did not influence the outcome in our series, similar to the experience of several recent studies.[17,19,23]

Donor type has been considered as a major factor influencing outcome following allogeneic SCT. Studies specifically addressing the role of SCT in children with MDS have indicated a probability of EFS of about 50% following transplant with an HLA-matched family donor,[15,23] the same as obtained in our series. The use of unrelated BM donors was associated with a higher incidence of acute and chronic GVHD, a higher TRM, and EFS probability of around 29%.[22,24] Parikh et al[19] recently reported encouraging data regarding the use of UCB as a stem cell source for children with primary and secondary MDS. This study showed 3-year cumulative survival of 60%, with a lower incidence of acute and chronic GVHD.

Relapse has been a major contributor to mortality in pediatric patients, with a statistically significant higher relapse rate in RAEB and RAEB-t cases as compared with RC. Our study is similar to other studies that showed no correlation between relapse rate and MDS-subtype. In addition to graft failure, infections secondary to immunosuppression from GVHD and its therapy were a major cause of NRM in our patients, as shown in other series.[15,19,23] Overall, quality of life for most patients was excellent with all but 1 of the 7 surviving patients having a performance score of greater than or equal to 90%.

In conclusion, children with MDS may achieve encouraging OS and EFS following allogeneic SCT. The presence of monosomy 7 did not adversely affect the outcome after transplantation. Graft failure and relapse were the main causes of treatment failure. However, methods to further improve outcome in patients with MDS undergoing allogeneic transplantation are needed to decrease the relapse rate. This could be achieved through serial post SCT analysis of chimerism to detect the development of mixed chimerism, which is a predictor of poor outcome, and the use of preemptive immunotherapy with donor lymphocyte infusions or discontinuation of the immunosuppressive therapy.[25,26] Tailoring the condition regimens to reduce toxicity, while maintaining efficacy, is definitely beneficial to this group of patients. The outcomes are best in younger patients, and transplant is thus recommended early in the course of the disease.

Authorship and Disclosures

All authors critically reviewed and approved the manuscript. The authors declare no competing financial conflicts of interest.

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