High transplant related mortality associated with Haematopoietic stem cell transplantation for paediatric therapy related acute myeloid leukaemia (t-AML). A study on behalf of the United Kingdom Paediatric Blood and Bone Marrow Transplant Group.

Adam Gassas [1], Ponni Sivaprakasam[1], Michelle Cummins[1], Patricia Breslin[1], Katharine Patrick[2], Mary Slatter[3], Roderick Skinner[3], Geof Shenton[3], Brenda Gibson[4], Sarah Lawson[5], Toni Petterson[6], Michael Potter [6], Beki James[7], Rachael Hough[8] , Prashant Hiwarkar[9], Ajay Vora[10], Paul Veys[10], Josu De La Fuente[11], Robert Wynn[9], and Persis Amrolia[10].

AFFILIATIONS:

[1]Department of Haematology and Oncology, Royal Hospital for Children, Bristol, UK; [2]Department of Haematology and Oncology, Sheffield Children's Hospital, Sheffield, UK; [3]Children's Haemopoietic Stem Cell Transplant Unit, Great North Children's Hospital, Newcastle upon Tyne, UK; [4]Department of Haematology and Oncology, Royal Hospital for Children, Glasgow, UK; [5]Department of , Haematology and Oncology, Birmingham Children's Hospital, Birmingham, UK; [6]Department of Haematology and Oncology, Royal Marsden Hospital, Sutton, UK; [7]Department of , Haematology and Oncolog, Leeds Teaching Hospital, Leeds, UK; [8]Department of Haematology and Oncology, University College of London, London, UK; [9]Department of , Haematology and Oncology, Royal Manchester Children's Hospital, Manchester, UK. [10]Department of Haematology and Oncology, Great Ormond Street Hospital for Children, London, UK.[11]Department of Haematology and Oncology, St Marys Hospital, Imperial College, London, UK

WORD COUNTS: Abstract: 97 Text: 1906 Table: 1 Figure: 1

Running Title: Stem cell transplantation in paediatric t-AML.

Key words: therapy related AML, allogeneic transplantation, children, outcome.

All authors have no conflict of interest to declare.

ADDRESS FOR CORRESPONDENCE:

Adam Gassas, MBChB MSc FRCP FRCPCH DCH

Consultant Paediatric Haem/Onc/BMT

Division of Haematology/Oncology/BMT

Bristol Royal Hospital for Children

Upper Maudlin Street

Bristol

BS2 8BJ

Tel 0117 342 0348

adam.gassas@UHBristol.nhs.uk

adam.gassas@nhs.net

ABSTRACT

Paediatric therapy related acute myeloid leukaemia (t-AML) is rare and the outcome is poor. While allogeneic haematopoietic stem cell transplantation (HSCT) is generally the accepted modality of treatment, data regarding salvage chemotherapy, remission induction, conditioning regimens, transplant related mortality and outcome is scarce. Between 2000-2016, 36 children with t-AML were treated in seven UK paediatric HSCT centres. The most common salvage protocol for remission induction was FLAG with or without idarubicin and 28 patients were in complete morphological remission prior to BMT. Only 12 patients survived (33%). Transplant related mortality (TRM) was the leading cause of death.

INTRODUCTION

Therapy related acute myeloid leukaemia (t-AML) is a well-recognized clinical subtype of AML. [1] t-AML occurring in adults is usually associated with inferior outcome compare with de novo AML. [2-4] Although more children now survive their first cancer, data on paediatric outcomes of t-AML is limited, outcome historically has been very poor (15-30%) and generally allogeneic HSCT is the treatment modality of choice. [5-7] Furthermore, it is not known whether the inferior outcome is due to disease relapse or transplant related mortality. Our aim herein was to retrospectively assess the outcome of children who were diagnosed with t-AML and received HSCT in a dedicated JACIE accredited paediatric UK transplant centre.

PATIENTS AND METHODS

A survey questionnaire was sent to all UK Paediatric HSCT centres asking centres to identify children (0-18 years) who were transplanted in their centres for a diagnosis of t-AML from January 2000 – January 2016. Definition of t-AML was according to the WHO criteria. [1] Electronic data collection for patient demographics, HSCT characteristics and outcomes were collected.

STATISTICAL ANALYSIS

SAS software was used to carry out the statistical analyses. Time to event (relapse or death) and the probability of event free survival (EFS) and overall survival (OS) for different endpoints was determined using Kaplan–Meier curves. The association between pairs of categorical variables was evaluated using Fisher's exact test. A two tailed p value of <0.05 was considered to be statistically significant. The cumulative incidence of relapse and treatment-related mortality was estimated taking into account the competing risks of other events. Given the small number of survivors, a Cox proportional hazard survival model was not possible and the impact of different variables on survival was descriptive. Transplant related mortality (TRM) was defined as any death in remission.

RESULTS

Seven UK paediatric HSCT centres participated in the study and sent data regarding children who received HSCT for t-AML. Total number of patients was 37, however, one child with t-AML and a previous diagnosis of neuroblastoma died within 2 days of the diagnosis of t-AML and did not receive HSCT and was therefore excluded from the study. Median time from the original diagnosis to the diagnosis of t-AML was 3.8 years (1-9.5 years).

Cytogenetics results were available in 30 patients and 23 of them had poor risk cytogenetics (p=0.02) including MLL rearrangements, monosomy 7 and complex karyotype (see Table 1). Fourteen patients had received radiotherapy as part of their treatment for first cancer. Fludarabine, high dose cytarabine, and granulocyte colony stimulating factor (FLAG) with or without idarubicin (FLAG-Ida) was the most common salvage regimen prior to HSCT. Remission status prior to HSCT was known in 28 patients, 24 were in complete remission (CR) prior to HSCT leading to a statistically significant likelihood to enter remission prior to HSCT (p=0.001). The majority (28 patients) received a graft from an unrelated donor (18 from an unrelated adult donor and 10 from a cord unrelated progenitor stem cell source). Conditioning regimens varied among centres – overall 28 patients received myeloablative regimens (TBI based in 8 patients) and 6 patients received reduced intensity conditioning. All patients received cyclosporine A (CSA) as GVHD prophylaxis. Recipients of unrelated

donor graft received campath 1H as serotherapy and recipients of cord progenitor stem cells received mycophenolate mofetil (MMF) along with CSA. A further agent for GVHD prophylaxis such as methotrexate or MMF was added to the CSA for mismatched unrelated grafts. Table 1 summarises patient and transplant characteristics.

SURVIVAL

One patient was lost to follow up, therefore, survival outcome was available on 35 patients. At a median follow up of 7.3 years (range 2.1 - 16.1) 12 patients are long term survivors (OS = 34%) and 23 patients have died. Thirteen out of the 23 patients (56%) died from TRM and 10 patients (44%) died from disease relapse giving an overall TRM rate of 37% and a relapse rate of 29%. All patients who relapsed died, therefore EFS and OS was the same (figure 1). Potential variables that had an impact on survival were as follows: 1. t-AML secondary to solid tumour vs t-AML secondary to haematological/lymphoid malignancy. 2. Donor source (living unrelated donor vs unrelated cord vs matched sibling donor). 3. Poor risk cytogenetics. 4. Conditioning regimens.

There were 17 patients with t-AML post a solid tumour and 11 of them died post HSCT and 12 out of the 18 patients with t-AML post haematological/lymphoid malignancy have died post HSCT. Eighteen patients received HSCT from a living UD and 11 died (TRM=5, relapse=6). Ten patients received unrelated cord progenitor stem cells and 8 died post HSCT (TRM=6, relapse=2). Eight patients received matched sibling donor and 4 died (TRM=2, relapse=2). Twenty-three patients had poor risk cytogenetics. Five out of nine patients with MLL gene rearrangement died, 3 from TRM and 2 from relapsed disease. Five out of nine patients with monosomy 7 died, 4 from TRM and 1 from relapsed disease. All patients with complex cytogenetics (n=5) died, 3 from TRM and 2 from relapsed disease. Therefore, 8 out of 23 patients with poor risk cytogenetics are alive compared to 4 out of 12 patients without poor risk cytogenetics (*P* = NS). As for the conditioning regimens; of 9 patients who were conditioned with fludarabine, treosulphan and thiotepa (FTT) or fludarabine and treosulphan (FT), 7 have died, 4 from TRM and 3 from relapsed disease. Of those who were conditioned with BU/CY/Mel (n=8), 5 have died, 4 from TRM and 1 from relapsed disease. Eight patients were

conditioned with CY/TBI and 4 have died, 2 from TRM and 2 from relapsed disease and 6 patients were conditioned with FLU/Mel and 4 have died 1 from TRM and 3 from relapsed disease. Those who did not achieve remission prior to BMT (n=4) 3 of them died post BMT, 2 from TRM and 1 from relapsed disease. Seventeen patients had acute GVHD, grade I in 4 patients (1 died from TRM), grade II in 6 patients (2 died from relapse and 2 died from TRM), grade III in 2 patients and both died from TRM and grade IV in 5 patients (2 died from TRM and 1 died from relapse). Therefore, 7 patients with GVHD died from TRM, 5 from multi organ failure and 2 from sepsis. The cause of TRM in the remaining 6 patients who did not have GVHD were as follows; pneumonitis with respiratory failure (n=3), adeno virus infection (n=1), pseudomans aeruginosa sepsis (n=1) and multi organ failure (n=1).

DISCUSSION

In this multi-centre retrospective paediatric study for t-AML and HSCT, we found that the majority of t-AML is associated with poor risk cytogenetics. However, remission induction and maintaining remission to proceed to HSCT was possible in the majority of patients. Furthermore, the main cause of death was TRM rather than relapse and a survival of 34% was similar to other published reports of t-AML. [3-8]

Among 2589 children with cancer treated at MD Anderson, Aguilera et al reported 22 children with t-AML who were treated differently with some subtle differences in their outcome. Three patients received supportive care only. Group 1 (n=5) underwent HSCT without induction chemotherapy. Group 2 (n=5) patients received AML-type chemotherapy and HSCT post remission (n=5). Group 3 (n=4) received HSCT as salvage therapy. The respective 2-year survival rates for groups 1, 2, and 3 were 20%, 40%, and 25% (P=0.85). [9] Due to the small number of patients there was no statistical significance difference in their outcome. However, group 2 which included patients who received AML-type chemotherapy followed by HSCT had the best outcome. In our study all patients with t-AML were treated following the same principle since post HSCT outcome is generally superior if

patients achieve good remission prior to HSCT and achieving remission prior to HSCT was possible in the majority of our patients.

t-AML is recognized as an independent poor prognostic factor among AML. [10] Nonetheless, in our study, the main cause of death was TRM rather than disease relapse. We acknowledge the fact that TRM is a competing risk for disease relapse and it is unknown how many of the patients who died from TRM would have relapsed if they lived longer. Woodard et al analysed the results of allogeneic HSCT in 38 children with t-AML to determine which factors, if any, affected outcome. Three-year OS and EFS were the same (15.4 +/- 5.8%) and a very high 3-year TRM (59.6 +/- 8.4%). [11] This high TRM incidence was similarly observed in our study. A large adult study analysed outcomes in 868 persons with t-AML (n=545) or t-MDS (n=323) receiving allogeneic transplants from 1990 to 2004. EFS and OS were 32% (95% CI, 29-36) and 37% (34-41) at 1 year and 21% (18-24) and 22% (19-26) at 5 years, respectively. In a In multivariate analysis, 4 risk factors had adverse impacts on EFS and OS: (1) age older than 35 years; (2) poor risk cytogenetics; (3) t- AML not in remission or advanced t-MDS; and (4) donor other than an HLA-identical sibling or a partially or well-matched unrelated donor. [12] In our paediatric study survival statistical model was not possible due to small number of patients.

While it is not possible to change the t-AML aggressive disease biology and high risk features, there may be some measures that could be taken to reduce TRM. We believe that this high TRM incidence was caused by the heavily pre-treated status of these patients since all patients received multiple cycles of chemotherapy for their original cancer and strong salvage chemotherapy for the t-AML, FLAG or FLAG/Ida. Furthermore, almost all patients received 2 cycles of FLAG prior to HSCT but the majority were in remission after the first cycle. Therefore, provided a suitable donor is available and the patient is in remission, proceeding to HSCT after one cycle of FLAG/Ida may lead to a reduction in TRM.

Another factor that may have contributed to the high incidence of TRM was the choice of the conditioning regimen. Our observation suggests the children who received a fully myeloablative

conditioning regimen such as BU/CY/Mel have a high TRM rate (50%), while those who received a reduced intensity conditioning regimen such as FLU/Mel had a high relapse rate (50%). It is possible that a reduced toxicity rather than a reduced intensity regimen such as BU/FLU may lead to a reduction of TRM while preserving anti leukaemic activity. [13-15]. Within the UK, we plan to investigate this approach prospectively.

We acknowledge the limitation of our study, in particular, the retrospective design and that survival model statistical analysis was not possible due to the limited number of patients. However, our observations suggests that TRM is the main cause of death in this very high risk paediatric malignancy and potential measures such as reduction of salvage chemotherapy after achieving remission and a careful choice of the conditioning regimen may be helpful in reducing TRM. Larger prospective studies are required.

Acknowledgements:

All authors are indebted to the patients and their families as well as to the nursing, medical staff in all UK Paediatric HSCT centers for their excellent patient care and continuing support.

All authors have no conflict of interest to declare.

REFERENCES

1. Sabattini E, Bacci F, Sagramoso C, Pileri SA. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues: an overview. Pathologica. 2010;102:83-7.
2. Kayser S, Döhner K, Krauter J, Köhne CH, Horst HA, Held G, et al. The impact of therapy-related acute myeloid leukemia (AML) on outcome in 2853 adult patients with newly diagnosed AML. Blood. 2011;117:2137-45.
3. Schoch C, Kern W, Schnittger S, Hiddemann W, Haferlach T. Karyotype is an independent prognostic parameter in therapy-related acute myeloid leukemia (t-AML): An analysis of 93 patients with t-AML in comparison to 1091 patients with de novo AML. Leukaemia. 2004;18:120-5
4. Granfeldt Østgård LS, Medeiros BC, Sengeløv H, Nørgaard M, Andersen MK, Dufva IH, et al. Epidemiology and Clinical Significance of Secondary and Therapy-Related Acute Myeloid Leukemia: A National Population-Based Cohort Study. J Clin Oncol. 2015;33:3641-9.
5. Sandoval C, Pui CH, Bowman LC, Heaton D, Hurwitz CA, Raimondi SC, et al. Secondary acute myeloid leukemia in children previously treated with alkylating agents, inte rcalating topoisomerase II inhibitors, and irradiation. J Clin Oncol. 1993;11:1039-45
6. Sandler ES, Friedman DJ, Mustafa MM, Winick NJ, Bowman WP, Buchanan GR. Treatment of children with epipodophyllotoxin-induced secondary acute myeloid leukemia. Cancer. 1997;79:1049-54.
7. Hale GA, Heslop HE, Bowman LC, Rochester RA, Pui CH, Brenner MK, et al. Bone marrow transplantation for therapy-induced acute myeloid leukemia in children with previous lymphoid malignancies. Bone Marrow Transplant. 1999 ;24:735-9.
8. Felice MS, Zubizarreta PA, Chantada GL, Alfaro E, Cygler AM, Gallego M, et al. Acute myeloid leukemia as a second malignancy: report of 9 pediatric patients in a single institution in Argentina. Med Pediatr Oncol. 1998;30:160-4

9. Aguilera DG, Vaklavas C, Tsimberidou AM, Wen S, Medeiros LJ, Corey SJ. Pediatric therapy-related myelodysplastic syndrome/acute myeloid leukemia: the MD Anderson Cancer Center experience. J Pediatr Hematol Oncol. 2009;31:803-11.

10. Larson RA. Is secondary leukemia an independent poor prognostic factor in acute myeloid leukemia? Best Pract Res Clin Haematol. 2007;20:29-37.

11. Woodard P, Barfield R, Hale G, Horwitz E, Leung W, Ribeiro R, et. Outcome of hematopoietic stem cell transplantation for pediatric patients with therapy-related acute myeloid leukemia or myelodysplastic syndrome. Pediatr Blood Cancer. 2006;47:931-5.

12. Litzow MR, Tarima S, Pérez WS, Bolwell BJ, Cairo MS, Camitta BM et al. Allogeneic transplantation for therapy-related myelodysplastic syndrome and acute myeloid leukemia. Blood. 2010 115:1850-1857.

13. Andersson BS, de Lima M, Thall PF, Wang X, Couriel D, Korbling M, Roberson S, et al. Once daily i.v. busulfan and fludarabine (i.v. Bu-Flu) compares favorably with i.v. busulfan and cyclophosphamide (i.v. BuCy2) as pretransplant conditioning therapy in AML/MDS. Biol Blood Marrow Transplant. 2008;14:672-84.

14. Andersson BS, de Lima M, Thall PF, Madden T, Russell JA, Champlin RE. Reduced-toxicity conditioning therapy with allogeneic stem cell transplantation for acute leukemia. Curr Opin Oncol. 2009;21 Suppl 1:S11-5.

15. de Lima M, Couriel D, Thall PF, Wang X, Madden T, Jones R, et al. Once-daily intravenous busulfan and fludarabine: clinical and pharmacokinetic results of a myeloablative, reduced-toxicity conditioning regimen for allogeneic stem cell transplantation in AML and MDS. Blood. 2004;104:857-64.

Figure 1 title/legend (Figure 1: Kaplan-Meier for 35 children with t-AML post HSCT).