Anti-mullerian hormone and Inhibin B after haemopoietic stem cell transplantation in childhood: a comparison of myeloablative, reduced intensity and treosulfan-based chemotherapy conditioning regimens.

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Running short title: Gonadal markers after HSCT with variable-intensity regimens

Summary

Serum concentrations of Anti-Mullerian Hormone (AMH) and Inhibin B, along with pubertal status, hormone replacement and pregnancy data were used to assess potential fertility in survivors of childhood HSCT after three chemotherapy-conditioning regimens of differing intensity. Of 428 patients transplanted at GOSH between 1990-2012 for leukaemia and immunodeficiency, 121 survived >1 year after a single HSCT conditioned with one of 3 regimens: Group A had a Treosulfan-based regimen (low-toxicity); Group B had Fludarabine/Melphalan (Flu-Mel) (reduced-intensity) and Group C had Busulphan/Cyclophosphamide (Bu-Cy) (myelo-ablative).

Mean age at HSCT and follow-up were 3.6 and 11.8 years. Mean length of follow-up was 9.9 years. The mean AMH SD scores were significantly higher in Group A (-1.047) and Group B (-1.255) than Group C (-1.543), suggesting that ovarian reserve was less impaired after Treosulfan and Flu-Mel than after Bu-Cy. The mean serum AMH concentration was significantly better with Treosulfan than with Flu-Mel or Bu-Cy and >1.0 microg/l. In males, mean Inhibin B SD scores were significantly higher in Group A (-0.506) than in Group B (-2.53) and Group C (-1.23) with the Flu-Mel group suffering the greatest impairment. In conclusion, a Treosulfan-based regimen confers a more favourable outlook for gonadal reserve than Flu-Mel or Bu-Cy in both sexes. Significantly higher values of Inhibin B after Bu-Cy than after Flu-Mel may reflect recovery over time.

Keywords: Treosulfan; Anti-Mullerian Hormone; Inhibin B; Stem Cell Transplantation; Fertility.

Introduction

Chemotherapy conditioning regimens before haemopoietic stem cell transplantation (HSCT) generally contain one or more alkylating agents avoiding some of the most severe late sequelae associated with total body irradiation (Brennan & Shalet, 2002; Leiper, 2002). However, alkylating agents are known to impair gonadal function and subsequent fertility in males and females and increase the risk of premature menopause in a dose-related manner in childhood cancer survivors (Green *et al*, 2010; Green *et al*, 2009, Sklar *et al*, 2006). Busulphan-containing myeloablative regimens in particular are recognized to cause severely impaired ovarian reserve and fertility in female survivors of childhood HSCT (Grigg *et al*, 2000; Sanders *et al,* 1996; Tenturier *et al*, 1998; Michel *et al*, 1997; Thibaud *et al*, 1998; Afify *et al*, 2000; Laporte *et al*, 2011; Borgmann-Staudt *et al*, 2012; Bresters *et al*, 2014).

The development of reduced-intensity conditioning (RIC) regimens, such as the combination of fludarabine with the alkylator melphalan (Flu-Mel), has resulted in less toxicity (Rao et al, 2005; Chiesa &Veys, 2012). Limited data in adult studies using RIC conditioning with Flu-Mel or other melphalan-based regimens, suggest better fertility outcomes in females with preservation of reproductive capacity despite significant gonadotoxic damage (Shimizu *et al*, 2012; Papageorgiou *et al*, 2012; Singhal *et al*, 1996; Jackson *et al*, 1997). There is a paucity of research in children. A previous study from our centre of a young population of 40 adolescents and adults of both sexes who had undergone RIC transplants with Flu-Mel during childhood showed a reduced impact of Flu-Mel on reproductive potential at least in females when

compared with a second group of 51 who had received a myeloablative combination of busulphan and cyclophosphamide (Bu-Cy) (Panasiuk *et al,* 2015). Two later studies have reported spontaneous commencement or recovery of menstruation in most females after RIC-HSCT in childhood with similar conditioning regimens (Madden et al, 2016; Fujino et al, 2019) Treosulfan originally used as an anti-neoplastic agent in ovarian carcinoma is increasingly used in paediatric practice in both malignant and non-malignant disease. It is a prodrug of a bifunctional alkylating agent with myeloablative and immunosuppressive properties but is associated with less systemic toxicity than busulfan (Ten Brink *et al*, 2014; Slatter *et al*, 2018; Levi *et al*, 2018). Thus treosulfan-containing regimens may be referred to as 'low-toxicity' (Greystoke *et al*, 2008; Slatter *et al*, 2011; Slatter *et al*, 2015). There are no long-term follow up studies evaluating treosulfan in humans though it appears that it may have a milder gonadotoxicity profile than busulphan in male but not in female mice (Levi *et al*, 2018).

Anti-Mullerian hormone (AMH), produced by the theca granulosa of the growing pre-antral and early antral ovarian follicles, reflects the 'reserve' or the remaining pool of primordial follicles in the ovary at any particular time from birth up to the menopause (Anderson, 2012, Broer *et al*, 2014). Serum AMH is low during childhood gradually rising to reach a plateau in the mid-twenties after which it declines to the menopause (Kelsey *et al*, 2011; Li Fong *et al*, 2012, Broer *et al*, 2014, Anderson, 2012). AMH measurement represents the ovarian reserve in all age groups and is independent of the menstrual cycle (Brougham *et al*, 2012; Anderson 2012; George *et al*, 2019).

lnhibin B, produced by Sertoli cells in the seminiferous tubules of the testis is positively correlated with spermatogenesis in adult males (Pierik *et al*, 1998). Whilst not replacing semen analysis as the gold standard, it is considered a suitable surrogate marker of gonadal damage in adults and children (Crofton *et al*, 2003, Chada et al, 2003; van der Kooi et al, 2019; Laporte 2011; van Beek et al, 2007; van Casteren et al, 2009; Pierik *et al*,1998). Serum levels reach a peak during puberty and are stable thereafter (Crofton *et al*, 2002; Chada *et al*, 2003). Since there is evidence that low levels of AMH and lnhibin B reflect chemotherapy damage even in the absence of any other signs of clinical or biochemical dysfunction, they are promising tools for the prediction of infertility in childhood cancer survivors (van Beek *et al*, 2007 a,b; Krawczuk-Rybak *et al*, 2013; Miyoshi *et al*, 2013; Laporte *et al*, 2011; Lunsford *et al*, 2014; Charpentier *et al*, 2014).

The current study compares the effect of Flu-Mel, Bu-Cy and treosulfan-based conditioning regimens in survivors of HSCT in childhood using estimation of AMH in girls and Inhibin B in boys as markers of gonadal reserve.

Methods and materials

Subjects studied were children, adolescent and young adult survivors of myeloid leukaemia and other haematological and immunodeficiency disorders who had received HSCT in childhood at GOSH between 1990 and 2012 with conditioning agents including treosulfan, Flu-Mel or Bu-Cy. Patients were transferred to adult follow-up services at 18 years of age. 428 were identified

from the GOSH HSCT database of which 326 were still alive. After exclusion of 130 (see Fig 1), 196 met the eligibility criteria of surviving one year or more after a single HSCT prepared with one of the 3 stated conditioning regimens. Fifty-six were lost to follow-up or domiciled abroad and 19 failed to consent or respond to the invitation leaving 121 participants in the study (Figure 1). Forty-one (25 males) received a Treosulfan-based regimen (Group A), 55 (32 males) received Flu-Mel (Group B) and 25 (13 males) received Bu-Cy (Group C). Patient and disease characteristics are shown in Table 1. Group C had a higher proportion of patients treated for leukaemia than groups A and B which consisted mainly of those treated for immunodeficiency disorders. Patients with acute myeloid leukaemia (AML) or myelodysplasia (MDS) or Haemophagocytic lymphohistiocytosis (HLH) had all received preceding treatment with standard chemotherapy protocols appropriate for the era of diagnosis (Hann *et al*, 1997; Gibson *et al*, 2011; Bergsten *et al*, 2017).

The study was a cross-sectional assessment of biochemical markers of fertility impairment comparing low-toxicity and RIC groups A and B with myeloablative group C and with normal age-matched control data. The majority of participants in Group B and C had formed the cohort previously described by Panasiuk et al (2015). The treosulfan-based low-toxicity group A was added and the other 2 updated to 2012 with the inclusion of children. Data were collected from March 2014 to June 2016.

Data extracted from the medical records included ethnicity; diagnosis; treatment; age at HSCT; type of HSCT; conditioning chemotherapy; puberty

onset; serial gonadotrophins; use of hormone replacement therapy and pregnancy details.

In children less than 18 years of age, two venous blood samples were obtained a year apart (T1 and T2) at routine clinic appointments, for the measurement of serum AMH in girls or lnhibin B in boys,. In patients of pubertal age (≥10 year), serum gonadotropins (LH and FSH) and sex steroids (oestradiol in girls and testosterone in boys) were measured in addition. Pubertal status was assessed by the method of Tanner at the same time as the venous sampling.

In those who had progressed to the adult follow-up services, a single sample of serum AMH or lnhibin B (S1), gonadotrophins and sex steroids was obtained. In those taking HRT at the time of the study we used the gonadotrophin concentration obtained at the time of adult reassessment of need for HRT. Gonadotrophin evaluation was omitted in any girl taking the oral contraceptive pill at the time of sampling. Detailed evidence of fertility and pregnancy outcome was sought by questionnaire.

Serum concentrations of LH, FSH, oestradiol and testosterone were estimated by routine assays from fresh samples. Serum for AMH and Inhibin B was separated, frozen and stored at -20 degrees C until required for assay. Inhibin B samples were assayed in 2 batches by the *Inhibin B Gen II ELISA Beckman Coulter* assay at GOSH. AMH was assayed in a single batch by the *AMH Gen II ELISA Beckman Coulter assay* at the Erasmus Medical Centre, Rotterdam where established normative data exists for children (Lie Fong *et*

al, 2012).

Measurements of AMH and lnhibin B were converted to standard deviation scores (SDS) using age-matched normal values and the mean SDS of Groups A, B and C compared. For statistical analysis values at T1, T2 and S1 were pooled. Values of analytes below the assay limit were allocated the value of the assay limit. Age-matched reference values for AMH were based on samples from a cohort of healthy girls assayed by the same method in the same laboratory (Lie Fong *et al*, 2012). Inhibin B concentrations in males up to 17 years were compared with age-matched reference values from previously published data (Crofton *et al*, 2002). In subjects >17 years of age reference data from 49 normospermic males from a subfertile population was used (Pierik *et al*, 1998). This was comparable to data evaluating inhibin B (median and 2.5-97.5 percentiles) in 307 healthy males from the general population (Andersson *et al,* 2004).

Mean (±SD) values of gonadotrophins in groups B and C were statistically compared but there were insufficient pubertal patients to do so in Group A.

Statistical analysis was conducted using GraphPad Prism v8 software. Data for continuous variables are presented as mean values with standard deviation. Between group statistical comparisons of parametric data were analysed with t-test. To compare non-parametric data, the U Mann–Whitney and Kolmogorov-Smirnov tests were used. Analysis of more then 2 groups of values was conducted by analysis of variance.

Ethics and Consent

The study was reviewed and approved by the Research Ethics Committee according to norms of research involving human subjects (REC reference 14/WA/0115). Patients were identified from the HSCT database and informed written consent obtained prior to data collection and blood sampling.

Results

The features of patients by treatment groups and chemotherapy doses are summarised in Table 1. Of 121 patients (70 male): 58% were from non-Caucasian ethnic backgrounds; 88 (73%) had HSCT for primary immunodeficiency; 13 (11%) for AML/MDS or CML; 18 (15%) for HLH. Groups A and B had significantly more patients with immunological disorders than group C.

For the whole cohort, the mean values for age at HSCT and at follow-up (at T1) and the length of follow-up were 3.63 (± 3.86), 11.81(± 0.94) and 9.89 (± 5.63) years respectively. The length of follow-up ranged from 1.8 to 23.8 years. Table1 includes these parameters and comparative statistics for the whole cohort and individual groups. Group A was significantly younger at HSCT than Group B and C (1.93 v 4.57 v 4.63 years respectively) but there was no difference between Groups B and C. In Group A the age at follow-up was also significantly younger (7.30 compared with 14.56 and 20.93 years respectively) and the length of follow-up shorter (5.39 compared with 10.11 and 16.57 years.) In addition, there was a significant difference between groups B and C for each parameter (Table 1).

Puberty and gonadotrophin data:

At the start of the study a total of 72 (60%) patients from all 3 groups had reached the age of 10 years or greater: 31 girls and 41 boys. Five of the boys (4 from Group A and 1 from Group B) remained pre-pubertal. The majority (83%) of children from group A had not reached age 10 years. In this group there was evidence of spontaneous puberty (≥ B2) in 1 female and (≥G2) in 2 male patients at T1 and a further 1 female 2 males a year later (at T2) giving a total in puberty of 2 and 4 respectively (Table 1). Gonadotrophin results from group A were within the normal range in both sexes but were too few for statistical analysis (Table 2).

Sixty- five (81%) patients from group B and C had reached age10 years, 30 females and 35 males (Table 1).

Girls: 20 girls entered puberty spontaneously; 17 of 18 (94%) of group B and 3 of 12 (25%) of group C. Ten girls required HRT for induction of puberty and one further for failed pubertal progression and premature ovarian failure. Thus 11 (37%) girls were treated with HRT in total: 1 from the group B (5%) and 10 (83%) from group C (Table 1). At the time of the study only 8/11 girls in Group C were still taking HRT.

The mean (± SD) values of FSH and LH with their ranges for the 2 groups are shown in Table 2. The mean FSH concentration was significantly higher in group C compared to group B (67.54 ± 50.63 v 6.95 ± 3.956 respectively). The mean LH value was also significantly higher (25.59 ± 16.47 v 4.8 ± 3.677 respectively) (Table 2).

Boys: Thirty-four boys ≥10 years age entered puberty spontaneously and none required hormonal therapy in either group. LH levels in pubertal boys

were in the normal range with no significant difference in mean values between Group B or C (5.566 ± 4.372 v 4.913 ± 3.403 respectively) (Table 2). The mean FSH concentration was significantly higher in Group B, than in group C (21.20 ± 11.96 v 7.747 ± 6.35 respectively).

*Offspring***:** Six Females, 3 in each group B and C, had 7 spontaneous pregnancies. All babies in the group B were live, healthy offspring but there was only 1 live birth in Group C. No males in the study had offspring but 2 of Group C had semen analysis showing normal counts.

AMH Results:

The mean AMH SDS for Groups A, B and C were negative indicating impaired ovarian reserve in all groups. Mean SDS for Group A (-1.047) was not significantly different from that of Group B (-1.255) but both were significantly different from Group C (-1.543) (Fig. 2, Table 2).

The mean serum AMH concentration for group A (1.59 ±1.83 µg/l) was significantly higher than that for Group B (0.73 ± 0.82 µg/l) which was also significantly higher than that of Group C (0.11 ± 0.027 µg/l) (Table 2).

Inhibin B results:

The mean Inhibin B SDS for all groups was negative. It was significantly higher in Group A (-0.506 ± 2.112) than in Group B (-2.53 ± 1.62) and in Group C (-1.23 ± 1.41) The mean Inhibin B SDS of Group B was significantly lower than that of Group C (Fig. 3, Table 2)

The mean Inhibin concentration was significantly higher in Group A (114.3 ±

90.01 pg/ml) than in Group B (46.78 ± 44.91 pg/ml) but no different from Group C (129 ±124.6 pg/ml) (Table 2). The mean value was significantly higher in Group C than in Group B. Inhibin B concentrations were more than -2SD below the mean in 12.8% of Group A, 71% of Group B and 40% of Group C.

Discussion

Much of the information regarding serum AMH and Inhibin B as sensitive markers of gonadal reserve in children and adults comes from the study of cancer survivors at various stages of treatment and follow-up (Laporte *et al*, 2011; Wigny *et al*, 2016; Morse *et al*, 2013; van Dorp *et al*, 2014; Lunsford *et al,* 2014; van der Kooi *et al*, 2019). Evaluation of serum AMH and Inhibin B concentrations in adult childhood cancer survivors has generally shown a decline in markers of gonadal reserve either in subsets or in the entire cohort compared with controls, particularly associated with some alkylating agents and abdominal radiation or TBI (Li Fong *et al*, 2009; Thomas-Tenturier 2015, Lunsford *et al*, 2014, Miyoshi *et al*, 2013, Charpentier *et al*, 2014; George *et al*, 2018; Krawczuk-Rybak *et al*, 2013; van Beek et al, 2007a,b; van den Berg et al, 2018). AMH and Inhibin B may be compromised even before treatment starts (van Dorp *et al*, 2014; Wigny *et al*, 2014; Brougham *et al*, 2012; van den Kooi *et al*, 2019). Despite a further decline with treatment recovery may occur, especially in the low-risk groups (van den Kooi *et al*, 2019; Brougham *et al*, 2012; Myoshi *et al*, 2016). However, the populations studied are not restricted to HSCT survivors and where they are included, they have often received irradiation or are studied in very small numbers (Jadoul *et al,* 2011; Laporte *et*

al, 2011; Thomas-Tenturier *et al*, 2015; Krawczuk-Rybak *et al*, 2013; Myoshi *et al*, 2016; Morse *et al*, 2013). Un-irradiated females who received busulphan pre-HSCT exhibited particularly impaired ovarian function clinically and associated with low AMH and other abnormal ovarian markers (Jadoul *et al*, 2011; Laporte *et al*,2011; Krawczuk-Rybak *et al*, 2013). Nonetheless, the low AMH concentrations were not usually significantly different from survivors with persisting ovarian function. Our current study, incorporating an additional low-toxicity treosulfan group, systematically compares the effect of three chemotherapy-conditioning regimens of different intensity in survivors of childhood HSCT for malignant and non-malignant disease, using AMH and Inhibin B as markers of gonadal reserve. Pre-pubertal children have been included.

The negative SD scores of both AMH and Inhibin B indicate compromised gonadal reserve in all treatment groups, although gender-specific differences are apparent. Amongst those in the myeloablative, Bu-Cy group (C) only 17% girls entered puberty spontaneously, and 41% required HRT for induction or continuing pubertal progression. The RIC Flu-Mel group (B) fared better, with 94% showing signs of spontaneous puberty and a single patient only requiring HRT. The serum gonadotrophin means lying within the normal range in this group are in contrast to the abnormally high pre-HRT gonadotrophins of the myeloablative group. The mean AMH SDS and the absolute AMH values were also significantly different indicating superior ovarian reserve in the Flu-Mel group. In adults, serum AMH ≤1.0 µg/l is regarded as an indicator of reduced fertility (La Marca *et al*, 2007; La Marca *et al*, 2015; Nelson *et al*, 2009) and ≤0.3 µg/l is an indicator of critically low ovarian reserve (La Marca

et al, 2010; Nelson *et al*, 2009) associated with menopausal transition (van Rooij *et al*, 2004; Sowers *et al*, 2008). Interpretation during childhood is more difficult, when low values may be normal in young children (Lie Fong *et al,* 2012, Kelsey *et al*, 2011). However, in both Flu-Mel and Bu-Cy conditioning, the mean AMH values were still less than 1 µg/l although particularly low at the limits of detection in the Bu-Cy group. These findings are not inconsistent with those of our previous paper. This suggested that girls in the Flu-Mel RIC group may have a greater window of opportunity for fertility judged by the longer time to FSH elevation but a significant proportion eventually became abnormal (Panasiuk *et al*, 2015). This notwithstanding, it is known that low serum AMH does not entirely preclude pregnancy, even with concentrations in the critically low range (Hamre *et al*, 2012; Dillon *et al*, 2013,). In the present study there were 7 spontaneous pregnancies in these 2 groups.

In the treosulfan group the follow-up is short and the patients are still very young. Nonetheless, those few of pubertal age showed spontaneous pubertal progression with normal gonadotrophins and without the need of hormonal intervention. The use of age-matched AMH SD scores in the 3 groups suggests that ovarian reserve after treosulfan, although somewhat reduced compared with normal control data, is significantly better than after Bu-Cy and at least as good as after Flu-Mel. It is notable that the mean AMH concentration in the treosulfan group is significantly higher than that in either of the other groups and is above the generally accepted cut-off level of 1 µg/l. Also AMH is still naturally rising during childhood. There is some evidence that AMH concentrations may be suppressed whilst the patient is taking the

oral contraceptive pill (Broer *et al*, 2014; Charpentier *et al*, 2014; van den Berg *et al*, 2010). Whilst a direct extrapolation cannot be assumed, HRT was commonly prescribed in the myeloablative group. The clinical symptomatology and biochemical parameters of this group however were in keeping with very low ovarian reserve. Our study does not take account of genetic variation in AMH that may also have bearing on ovarian reserve and age at menopause (Van Dorp *et al*, 2013, Kevenaar *et al*, 2007).

The onset of puberty was spontaneous in all boys of pubertal age in all three groups although there were few of this age in the Treosulfan group. The mean LH was normal in all groups and none required testosterone replacement clinically. Testosterone was lower after Flu-Mel than Bu-Cy likely to be related to the younger age of the former group.

The mean Inhibin B SD score was negative in each group indicating Sertoli cell impairment but it was significantly higher in the treosulfan group than in either of the other two. This suggests better fertility potential associated with treosulfan use. The most damage seems to have occurred in the Flu-Mel group, which is contrary to the findings of our previous study of a similar cohort. In this there was no difference in time to abnormal FSH elevation from the onset of puberty between the RIC Flu-Mel and the myeloablative Bu-Cy groups in males (Panasiuk *at al*, 2015). In our present study the Inhibin B SDS was significantly lower in the Flu-Mel group than the Bu-Cy group. In addition the mean Inhibin B concentration was exceptionally low in the former group whilst it was not significantly different between the other two groups. In fact 71 % of the subjects had Inhibin B concentrations lower than -2SD from

the mean in the Flu-Mel group in contrast to 13 % and 40% respectively in the treosulfan and the Bu-Cy groups. The explanation for this discrepancy may be two-fold. Apart from comparing different parameters, a significant fall in recruitment in the myeloablative group may be a confounding factor. Secondly, it is possible that recovery of spermatogenesis may have occurred due to extended follow-up in the myeloablative group. Van Dorp et al (2013) showed that recovery could occur over time if the initial inhibin B was greater than 100 ng/ml in adult males but negligible if less than 60 ng/ml. Rovo et al (2006) revealed recovery of spermatogenesis with longer follow up, younger age at HSCT and absence of GVHD and Anserini et al (2002) observed recovery of spermatogenesis in 50% of those conditioned with Bu-Cy. In the present study no patient has fathered a child but 2 Bu-Cy survivors are known to have normal semen analysis.

The study of HSCT late effects is naturally affected by the evolution of therapeutic regimens. This is reflected in the current study by the disparate ages and length of follow up in the 3 groups with the treosulfan group being significantly younger at HSCT and follow up, and having the shortest length of follow up. A greater proportion of patients had immunodeficiency disorders with little prior treatment and fewer were of pubertal age. Those conditioned with Bu-Cy formed the oldest group at time of the study with the longest follow up. The composition was mainly of young adults treated for AML/MDS prior to HSCT in childhood. However, gonadal function and fertility expectation is good following chemotherapy alone in AML and other types of leukaemia and cancers were excluded from this study (Liesner *et al*, 1994; Molgaard-Hansen

et al, 2013). The extent of ovarian insufficiency is related to age at which the gonadotoxic insult occurs with those of older age being more vulnerable (Jadoul et al, 2011; Borgmann-Staudt et al, 2012, Bresters et al, 2014). Although the Treosulfan group was significantly younger at HSCT, it is unlikely that this accounts for its favourable outcome, as the mean age of each group was under 5 years. We circumvented the problem of disparate ages at the time of the study by using mean AMH and Inhibin B SDS calculated from an age-matched population of normal children. However, the inequality in length of follow up may remain relevant to the different outcomes such as recovery.

In conclusion, although gonadal reserve is impaired in all groups of HSCT survivors who have received conditioning regimens of varying intensity, a treosulfan-based combination appears significantly less gonadotoxic than myeloablative Bu-Cy in both sexes. In girls, the mean AMH value lies in the normal range suggesting that the low toxicity profile of treosulfan may extend to improved chances of future fertility.

The impact of a Flu-Mel regimen, like Bu-Cy, appears to be gender specific. It is less gonadotoxic than Bu-Cy and comparable to treosulfan in girls, whilst causing the most severe impairment in boys. Recovery of spermatogenesis, after prolonged follow-up in the myeloablative group, may explain some of the difference but it is unknown if boys in the Flu-Mel group will follow a similar pattern when further time has elapsed.

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Alison Leiper conceived and designed the research study, recruited participants and performed the research, supervised the contribution from Maite Houwing, and wrote the paper.

Maite Houwing set up the research database, helped perform the research and carried out a preliminary analysis of the data.

Kanchan Rao played a key role in identification of patients for the study and ensuring complete follow up. She contributed essential SCT expertise and reviewed the manuscript.

Graham Davies facilitated the research in the immunology clinic at GOSH by imparting research information. He reviewed the manuscript.

Siobhan Burns enabled the research at the Royal Free Hospital, NHS Trust by informing and obtaining consent from the adult participants and ensuring research sample collection. She reviewed the manuscript.

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