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IMPACT OF T-CELL DEPLETION STRATEGIES ON OUTCOMES FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR IDIOPATHIC APLASTIC ANEMIA: A STUDY ON BEHALF OF THE EUROPEAN BLOOD AND MARROW TRANSPLANT (EBMT) SAA WORKING PARTY

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Summary

We retrospectively analyzed the outcomes of 1837 adults and children with severe aplastic anemia (SAA) who underwent matched sibling donor (MSD) and matched unrelated donor (MUD) haemopoietic stem cell transplantation (HSCT) between 2000 and 2013. Patients were grouped by transplant conditioning containing either ATG (n=1283), alemtuzumab (n=261) or no serotherapy (NS) (n=293). The risks of chronic GvHD were significantly reduced when ATG or alemtuzumab were compared to no serotherapy (p=0.021 and p=0.003, respectively). Acute GVHD was significantly reduced in favor of alemtuzumab compared to ATG (P=0.012) and no serotherapy (p < 0.001). By multivariate analysis, when compared to ATG, alemtuzumab was associated with a lower risk of developing acute (OR 0.262; 95% CI 0.14-0.47; p<0.001) and chronic GVHD (HR 0.58; 95% CI 0.35 – 0.94; p=0.027). OS was significantly better in ATG and alemtuzumab patients compared with no serotherapy (p=0.010 and p=0.025). Our data shows inclusion of serotherapy in MSD and MUD HSCT for patients with SAA reduces chronic GVHD and provides a survival advantage over patients not receiving serotherapy. Notably, alemtuzumab reduced the risk of acute and chronic GvHD compared to ATG and indicates that alemtuzumab might be the serotherapy of choice for MSD and MUD transplants for SAA.

Key words

Transplantation

Severe aplastic anemia (SAA)

Anti-thymocyte globulin (ATG)

Alemtuzumab

Graft vs host disease (GVHD)

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Introduction

Haemopoietic stem cell transplantation (HSCT) from a matched sibling donor (MSD) is the treatment of choice for children and young adults with idiopathic severe aplastic anemia (SAA), with overall survival rates of 75-100% [1-6]. Moreover, HSCT from matched unrelated donors (MUD) represent the best second-line treatment option for SAA patients failing initial immunosuppression irrespective of age (if <60) [7,8]. However, current problems remain graft failure (4-14%), acute GvHD (11-18%) and chronic GvHD (30-40%) [9,10]. Chronic GvHD significantly impairs both quality of life and overall survival following HSCT [11]. Risk factors for development of chronic GvHD include prior acute GvHD, use of peripheral blood as a source of stem cells, the use of a female donor for male recipients, mismatched and unrelated donors and older patient age [12]. The occurrence of GvHD following HSCT for SAA offers no advantage for the patient, since no benefit from the graft versus leukemia (GVL) effect is expected, as seen following HSCT for acute leukemia.

Until recently it was unclear whether serotherapy should be included in the conditioning regimen to negate the risk of GvHD, without increasing the risk of graft failure, infection or compromising overall survival. Indeed, the only prospective randomized trial to date which attempted to address this issue, failed to show any survival benefit from the addition of Anti-Thymocyte Globulin (ATG) in the conditioning regimen, possibly due to slow accrual of patients and the study being inadequately powered [9]. This study also showed no difference in the incidence of acute and chronic GvHD rates between T cell replete and ATG containing regimens. In contrast, a more recent EBMT study showed that serotherapy using ATG did significantly benefit overall survival for MSD and MUD HSCT performed for SAA [13]. However, the optimal in vivo T cell depletion regimen is not known with current guidelines from EBMT and British Committee for Standards in Haematology (BCSH) suggesting either ATG or Alemtuzumab as possible options [7,14]. Recent reports from the UK using alemtuzumab as serotherapy in the conditioning regimen in both MSD and MUD transplantation for SAA have shown remarkably low rates of chronic GvHD compared to historical series [15,16]. However, previous attempts to determine the optimal serotherapy regimen in SAA have been hampered by small numbers [2,9]

In order to investigate the optimal serotherapy regimen for HSCT for idiopathic SAA and its impact on main transplant outcomes, we retrospectively analysed a large series of adults and children who underwent MSD and MUD HSCT with idiopathic SAA who were reported to European Bone Marrow Transplantation (EBMT) Society between 2000 and 2013 using the EBMT database. The goal of the study was to identify the serotherapy regimen with the lowest rate of GvHD, which is a surrogate of long-term morbidity and mortality after HSCT.

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METHODS

Patients

As per EBMT procedures (www.ebmt.org), this retrospective study was approved by the Severe Aplastic Anemia Working Party (SAAWP) and utilized data submitted with patient consent from 285 centers reporting to the EBMT database via the EBMT software (Promise) in the period 2000-2013.

Eligible patients were those with SAA who received a MSD or MUD SCT between 2000 and 2013. No selection was done regarding the conditioning regimen. However, only those undergoing first allogeneic transplants were included. Patients receiving cord blood as a source of stem cells and mismatched donors with one difference at the antigenic and/or allelic level in HLA-A, -B, -C, -DRB1 were excluded.

Patients with inherited bone marrow failure syndromes were not included. The diagnosis of SAA was made using established definitions [17-19]. Donor matching was performed for HLA-A, -B, -C, DRB1 and DQB1. Patients received chemotherapy or TBI-based conditioning with either ATG or alemtuzumab as serotherapy or no serotherapy (NS) prior to transplantation. The source (horse or rabbit) and dosing schedule of ATG, and details of immunosuppression were not available for analysis.

Study Endpoints

The primary study endpoint was the risk of chronic GvHD. Any grade of GvHD from day 100 was included. Secondary endpoints included overall survival, graft failure and acute GvHD (grades II-IV). Event free survival (EFS) was defined as the absence of engraftment, graft loss, second transplant, malignancy following transplant, relapse and death. Diagnosis of acute of chronic GvHD was made by individual centres.

Statistical analysis

Comparisons of characteristics between treatment groups were made using the $\chi 2$ test for categorical data and Mann-Whitney or Kruskal-Wallis test for continuous variables. Overall Survival was defined as the time from allogeneic transplantation to death from any cause, with patients still alive at their last follow up being censored. Event Free Survival was defined as the time from transplant until the first event among graft failure, graft loss, second transplant, second malignancy, relapse or death; cases still at risk were censored at their last follow up. Probabilities of OS and EFS were computed using Kaplan-Meier estimator and compared by the Log-Rank test. Chronic GvHD was analyzed in a competing risk framework, with any of the failures included in EFS considered as competing event, and performing a landmark analysis including only patients who survived event-free up to 100 days after transplant. The cumulative incidence of chronic GvHD was calculated by the proper non-parametric estimator and comparisons were made using the Gray test. The same competing event were considered for the development of acute GvHD, restricting to the first 100 days. Since several dates of onset were missing, the occurrence of acute GvHD was

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analyzed comparing percentages of cases and of competing events occurred by the day 100 between the three groups of treatment. Adjusted comparisons of endpoints between treatment groups considered the following factors: age at transplant (as continuous variable), donor type (MSD versus MUD), gender mismatch (Male patients-Female donor versus all other combinations), stem cells source (peripheral blood versus bone marrow) and conditioning regimen (with TBI versus without TBI). Adjusted effects on overall survival, event free survival and chronic GvHD incidence were estimated in terms of hazard ratios using the Cox model with a shared random center effect ("frailty model") to take into account unmeasured confounders related to belonging to the same center. Modification of the effect of the conditioning treatment in subgroups was explored including interaction terms.

Adjusted effects on the occurrence of acute GvHD were estimated in terms of Odds Ratios using multinomial logistic regression, results are reported in terms of the Odds Ratio (OR) of developing acute GvHD rather than being event-free at day 100: OR>1 indicates a risk factor for acute GvHD while OR<1 indicates a protective factor. All p-values shown are from two-sided tests and the reported confidence intervals (CI) refer to 95% boundaries. A significance level 5% was considered for all tests, except that for interactions, were 10% was applied.

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Results

The characteristics of the patients and their transplantations are summarized in Table 1. A total of 1837 patients were eligible for analysis: 1283 patients received ATG, 261 patients received alemtuzumab and 293 patients received no serotherapy in their transplant conditioning. The median age was 21.7 years (range 0.3- 76.8). 1155 patients received transplants from a MSD and 682 from a MUD

In the ATG and no serotherapy group the commonest conditioning regimen was cyclophosphamide alone, in contrast to the alemtuzumab group which more commonly received fludarabine/cyclophosphamide (65.5%). More patients received alemtuzumab in their conditioning regimen as part of a MUD transplant than a MSD transplant (73.9% vs 26.1%). This finding was reversed in the ATG and no serotherapy group.

Hematopoietic recovery

In each of the three treatment groups, patients were categorized as having engrafted, or as having primary or secondary graft failure. Overall, 1617 of 1816 (89%) patients successfully engrafted. Failure to engraft (i.e. the sum of primary and secondary graft failure) was not significantly different between the ATG, alemtuzumab and no serotherapy groups: 11.3%, 9.6% and 10.7% respectively (p=0.732).

Acute and Chronic GvHD

Observed rates of grade 2-4 acute GvHD in the ATG, alemtuzumab and no serotherapy group were 13.3%, 6.7% and 19.1% respectively (P<0.001). This difference in the observed rates of acute GvHD was significantly reduced in favor of alemtuzumab compared to ATG (P=0.012) and no serotherapy (p < 0.001). Other variables associated with a higher probability of developing acute GvHD included MUD transplant and use of peripheral blood (PB) as source of stem cells: adjusted OR were 2.79 (p<0.001) for MUD versus MSD transplantation and 1.49 (p= 0.011) for PB versus BM source. By multivariate analysis, when compared to ATG, alemtuzumab was associated with a lower risk of acute GvHD (OR 0.262; 95% CI 0.14-0.47; p<0.001). Compared to ATG, receiving no serotherapy led to a higher probability of developing acute GVHD (OR 1.647; 95% CI 1.12-2.41; p=0.010), whereas the use of alemtuzumab significantly reduced the risk of acute GvHD compared to NS (OR 0.159; 95% CI 0.08-0.31, p=0.000).

Figure 1 (a) shows the cumulative incidence (CI) of chronic GvHD. One thousand, two hundred and thirteen patients (N=1213, 66%) were evaluable for analysis: fifteen percent of patients (N=283,) were not considered at risk of chronic GvHD as they did not reach day 100 alive and event free. Missing information on chronic GvHD accounted for a further 341 (19%) cases. The 5-year cumulative probability of chronic GvHD in ATG, alemtuzumab and no serotherapy groups were 22.0% (95% CI 19.0 -25.1), 16.9% (95% CI 10.9-22.8) and 30.4% (95% CI 24.0- 36.8) respectively (p=0.006). The risks were significantly reduced when either ATG or alemtuzumab were compared to no serotherapy (p=0.021 and p=0.003),

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respectively. Between the two serotherapies, the difference in favor of alemtuzumab was borderline (p=0.083). All factors previously identified as associated with chronic GvHD, (age, donor type, gender mismatch, source and conditioning regimen - TBI yes vs no) retained a significant association with chronic GvHD in multivariate analysis (Table 2). After adjustment for these other covariates, alemtuzumab was associated with a significantly lower rate of chronic GvHD in comparison to both no serotherapy transplants (HR 0.43; 95% CI 0.25 – 0.76; p =0.004) and ATG transplants (HR 0.58; 95% CI 0.35-0.94; P=0.027).

Overall survival

The 5-year cumulative probability of OS in the ATG, alemtuzumab and no serotherapy groups was 80.2% (95% CI 77.7-82.7 %), 81.5% (95% CI 76.1-86.8%) and 72.5% (95% CI 67.1-78.0%) respectively (p=0.021) (Figure 1, b). OS was significantly better in both ATG and alemtuzumab patients compared with no serotherapy (p=0.010 and p=0.025 respectively). However, comparison between ATG and alemtuzumab failed to reach significance (p=0.604). Factors significantly associated with OS in multivariate analysis included age, donor source and type of serotherapy (Table 3). In multivariate analysis, alemtuzumab was associated with a significantly increased OS in comparison to no serotherapy transplants (HR 0.48; 95% CI 0.32-0.74, p=0.001) and a trend towards significance against ATG transplants (0.73; 95% CI 0.51-1.04, p=0.081).

Event free survival

There was no difference in the rates of EFS across the three groups: the cumulative probability of achieving 5-year event free survival was 71.8% in the ATG group (95% CI 68.9-74.6), 73.2% in the alemtuzumab-treated group (95% CI 66.9-79.4) and 68.3% in the group that received no serotherapy (95% CI 62.6-74.0; p=0.393) (Figure 1, (c)).

Causes of Death

Causes of death are categorized in Table 4 (Supplementary material). In all treatment groups, infection was the most common cause of death and accounted for 30.7-47.6% of deaths. There was no significant difference between the causes of death across the different types of serotherapy (p=0.131).

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Discussion

The present study is the largest analysis to document transplant outcomes after in vivo T- cell depletion (alemtuzumab or ATG) for adults and children with idiopathic SAA compared with T-cell replete HSCT. Retrospective data suggests that ATG improves survival [13]. However, the optimal strategy for in-vivo T-cell depletion is uncertain. This is of particular importance as GvHD remains the most unwanted complication after HSCT in AA; furthermore chronic GVHD is a major factor in reducing long term survival in SAA HSCT survivors [20]. Thus measures which prevent chronic GVHD in SAA HSCT are urgently required. Our analysis indicates that patients with acquired SAA who receive T-cell depletion with either ATG or alemtuzumab as part of their HSCT conditioning, have significantly lower risks of acute and chronic GvHD, and a survival advantage when compared to patients receiving no serotherapy. The lower GvHD rates seen with in vivo T-cell repletion were not offset by higher graft failure or lower EFS. Crucially, alemtuzumab significantly reduced rates of acute GvHD compared to ATG. By multivariate analysis, alemtuzumab was associated with a significantly lower rate of chronic GvHD compared to ATG-based transplantation and receiving no serotherapy. Taken together, these data suggest that alemtuzumab should be the preferred agent of choice for serotherapy in MSD and MUD HSCT in SAA

This reduction in GvHD is highly desirable in view of the associated morbidity and mortality for the patients, with an associated increase in their quality of life [11,20]. There are impacts for health services as chronic and severe GvHD is associated with an increased hospital stay. Our study has some limitations. The choice of treatment strategy (including the schedule, and dose of *in vivo* T cell depletion), diagnosis and management of chronic GvHD was decided by individual centres. Nevertheless, this is the largest study to date examining outcomes following in vivo T-cell depletion with ATG, alemtuzumab and T cell replete HSCT in SAA. Furthermore, in order to counter the inherent biases, we performed controlled comparisons of T cell replete and deplete with known prognostic factors. We recognize our study period spans 13 years, but when year of transplant was considered both as a continuous variable and categorical (2000-2005 vs 2006-2013), there was no difference in each of the outcomes. Age at transplantation was highly significant for every endpoint by multivariate analysis, showing an average increase of risk by 2-3% for every additional year of age.

These data are in agreement with those reported by other groups, consistent with similarly low rates of chronic GvHD with alemtuzumab-based regimens; and this is associated with stable mixed T-cell chimerism that persists on withdrawal of post graft immunosuppression [15,16,20].

The superior outcomes for acute and chronic GvHD seen with alemtuzumab compared to ATG could potentially be explained by a greater T-cell depletion. Whereas historically this would have led to a greater incidence of infections due to delayed immune reconstitution, this may no longer be the case because of advances in supportive care. Infection was the most common cause of death, with a trend for more infections in patients receiving serotherapy vs no serotherapy. There is a concern that alemtuzumab may increase the risk of viral infections

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given its marked T-cell depleting properties. However, this risk was not higher using alemtuzumab compared to ATG, and does not appear to be higher compared with historical data using no serotherapy [12]. The persistence of recipient CD8+ T-effector cells post fludarabine, cyclophosphamide, and alemtuzumab (Campath-1H) (FCC) HSCT for SAA that explains the mixed T-cell chimerism, may confer a degree of anti-virus immunity, since the incidence of CMV disease was low in a UK single center study [21].

The OS observed in this study is broadly in-line with outcomes seen over this time period (2000-2015). We propose the predominant reason for the OS being 10% higher than EFS is salvage with a second transplant if graft failure of relapse occur. The GvHD/relapse free survival (GRFS) is becoming more frequently used to track transplant outcome [22]. The use of this composite endpoint may be the most appropriate indicator to monitor transplant outcomes following HSCT for SAA and its use should be considered for future studies.

A recent retrospective review of 833 patients receiving either Horse or Rabbit ATG following HSCT for SAA, concluded that rabbit ATG rather than horse ATG should be used for the HSCT conditioning regimens for SAA [23]. This was based on higher rates of acute and chronic GvHD when using horse ATG compared to rabbit ATG. In our data set, the subtype of ATG preparation was not collected. However, we would suggest that in the interim until a comparison is performed between alemtuzumab and rabbit ATG, due to the superiority of alemtuzumab over ATG in this analysis, alemtuzumab is the preferred serotherapy of choice.

Whilst a randomized prospective study would have been preferable our data has important implications for clinical practice. We have shown that inclusion of serotherapy in MSD and MUD HSCT for patients with SAA reduces acute and chronic GvHD, and provides a survival advantage over patients not receiving serotherapy. These findings confirm that in vivo T cell depletion should be used for MSD and MUD HSCT for SAA. Our data also suggest that alemtuzumab is the preferred serotherapy agent of choice for both MSD and MUD transplants for SAA.

Author Contributions

SS, JM, CM and AR designed the study with input from all other authors. CK assembled data from the registry with all other authors. SI and GS analyzed the data, which was interpreted by all other authors. KC and SS wrote the manuscript, which was reviewed, modified and approved by all other authors.

Conflict of Interest Statement

We declare no competing interests.

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Table 1 Patient and transplantation-related characteristics.

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	ATG	Alemtuzumab	No serotherapy	P-value
No of patients	1283 (69.8%)	261 (14.2)	293 (15.9%)	
Sex, n (% of treatment group)				
Male	717 (55.9%)	144 (52.2%)	142 (48.5%)	0.069
Female	566 (44.1%)	117 (44.8%)	151 (51.5%)	
Age at transplantation, y (% of treatment group)				
<15 years	368 (28.7%)	79 (30.3%)	58 (19.8%)	0.003
≥15 years	915 (71.3%)	182 (69.7%)	235 (80.2%)	
Transplant type, n (% of treatment group)				
Matched sibling	852 (66.4%)	68 (26.1%)	235 (80.2%)	<0.001
Matched unrelated	431 (33.6%)	193 (73.9%)	58 (19.8%)	
Conditioning regimen , n (% of treatment group)				
Cyclophosphamide	673 (52.5%)	28 (10.7%)	159 (54.3%)	<0.001
Fludarabine /Cyclophosphamide	259 (20.2%)	171 (65.5%)	59 (20.1%)	
Other	143 (11.1%)	38 (14.6%)	27 (9.2%)	
TBI - based	208 (16.2%)	24 (9.2%)	48 (16.4%)	
Year serotherapy received (% within year group)				
2000-2005	289 (66.1%)	44 (10.1%)	104 (23.8%)	<0.001
2006-2013	994 (71.0%	217 (15.5%)	189 (13.5%)	
Source of stem cells (% of treatment group)				
Bone marrow	912 (71.1%)	153 (58.6%)	174 (59.4%)	<0.001
Periperal blood	371 (28.9%	108 (41.4%)	119 (40.6%)	
Gender match between recipient and donor, n (% of treatment group)				
Gender mismatch (male patient, female donor)	277 (21.6%)	40 (15.3%)	70 (23.9%)	0.034
Gender matched (any other combination)	1006 (78.4%)	221 (84.7%)	223 (76.1%)	
Recipient-donor CMV status, n (% of treatment group)				
Neg/Neg	294 (25.7%)	112 (45.7%)	53 (20.2%)	<0.001
Neg/Pos	96 (8.4%)	23 (9.4%)	20 (7.6%)	
Pos/Neg	266 (23.3%)	43 (17.6%)	41 (15.6%)	
Pos/Pos	488 (42.7%)	67 (27.3%)	148 (56.5%)	
Median follow- up in months (range)	34.0 (0.7-178.2)	30.9 (1.1-134.4)	47.9 (1.8- 167.0)	<0.001

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Table 2 Multivariate analysis of chronic GvHD

HR (95% CI, p-value)	Chronic GVHD
Alemtuzumab vs ATG	0.58 (0.35- 0.94, p=0.027)
ATG vs no serotherapy	0.75 (0.53-1.07, p=0.108)
Alemtuzumab vs no serotherapy	0.43 (0.25-0.76, p=0.004)
Age	1.02 (1.01-1.03, p<0.001)
Unrelated donor vs HLA identical sibling	2.01 (1.44-2.79, p<0.001)
Gender mismatch*	1.59 (1.19-2.13, p=0.002)
PBSC vs BMSC	1.93 (1.43-2.60, p<0.001)
TBI vs No TBI	1.52 (1.05-2.21, p=0.027)

HR Hazard Ratio, 95% confidence interval, p-value. Age, continuous, effect associated to +1 year of age. *Gender mismatch=male patient & female donor vs other combinations

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Table 3 Multivariate analysis on Overall Survival

HR (95% CI, p-value)	Overall survival
Alemtuzumab vs ATG	0.73 (0.51-1.04, p=0.081)
ATG vs no serotherapy	0.67 (0.50-0.88, p=0.005)
Alemtuzumab vs no serotherapy	0.48 (0.32-0.74, p=0.001)
Age	1.03 (1.02-1.04, p<0.001)
Unrelated donor vs HLA identical sibling	1.84 (1.45-2.34, p<0.001)

HR Hazard Ratio, 95% confidence interval, p-value.

Table 4 Causes of death by serotherapy

	ATG	Alemtuzumab	No serotherapy
No of deaths	(n=223)	(n=42)	(n=75)
Rejection/graft failure	58 (26.0%)	8 (19%)	15 (20.0%)
GvHD	27 (12.1%)	5 (11.9%)	17 (22.7%)
Infection	95 (42.6%)	20 (47.6%)	23 (30.7%)
Organ damage/failure	7 (3.1%)	1 (2.4%)	4 (5.3%)
Other	36 (16.1%)	8 (19.0%)	16 (21.3%)

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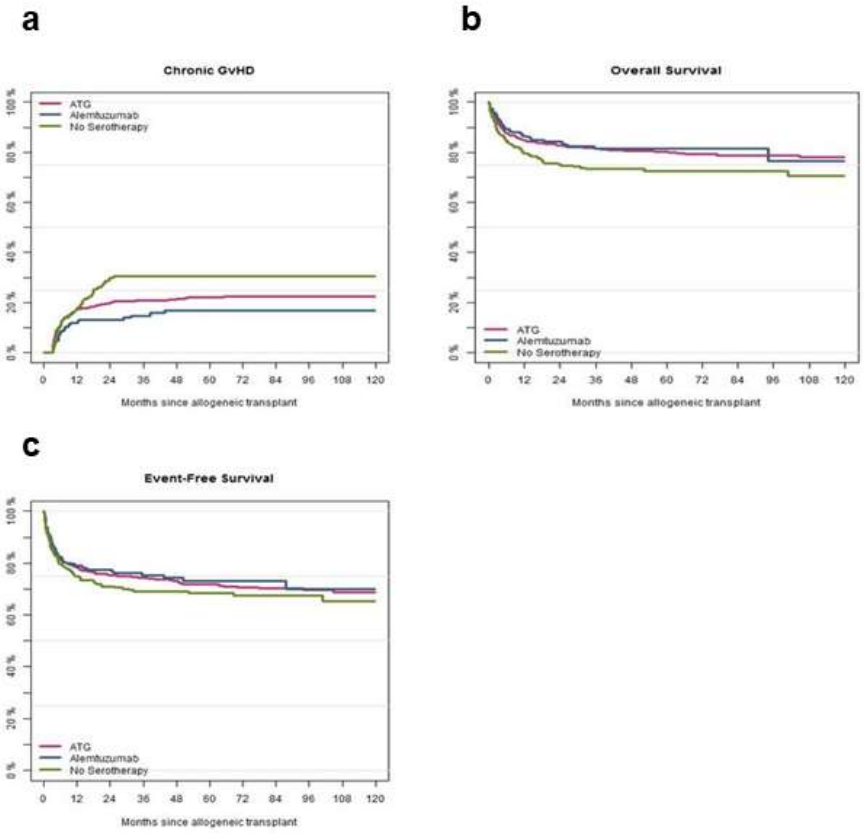

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