Syngeneic transplantation in aplastic anemia: pre-transplant conditioning and peripheral blood are associated with improved engraftment: an observational study on behalf of the Severe Aplastic Anemia and Pediatric Diseases Working Parties of the European Group for Blood and Marrow Transplantation

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ABSTRACT

Aplastic anemia is usually treated with immunosuppression or allogeneic transplant, depending on patient and disease characteristics. Syngeneic transplant offers a rare treatment opportunity with minimal transplant-related mortality, and offers an insight into disease mechanisms. We present here a retrospective analysis of all syngeneic transplants for aplastic anemia reported to the European Group for Blood and Marrow Transplantation. Between 1976 and 2009, 88 patients received 113 transplants. Most transplants (n=85) were preceded by a conditioning regimen, 22 of these including anti-thymocyte globulin. About half of transplants with data available (39 of 86) were followed by posttransplant immunosuppression. Graft source was bone marrow in the majority of cases (n=77). Transplant practice changed over time with more transplants with conditioning and anti-thymocyte globulin as well as peripheral blood stem cells performed in later years. Ten year overall survival was 93% with 5 transplant-related deaths. Graft failure occurred in 32% of transplants. Risk of graft failure was significantly increased in transplants without conditioning, and with bone marrow as graft source. Lack of posttransplant immunosuppression also showed a trend towards increased risk of graft failure, while anti-thymocyte globulin did not have an influence. In summary, syngeneic transplant is associated with a significant risk of graft failure when no conditioning is given, but has an excellent long-term outcome. Furthermore, our comparatively large series enables us to recommend the use of pre-transplant conditioning rather than not and possibly to prefer peripheral blood as a stem cell source.

Introduction

Aplastic anemia (AA) is a rare and life threatening disease for which treatment has greatly improved over the past decades. While response to immunosuppressive treatment has implied autoimmune causes, a stem cell defect might also play a role.[1] More recently, defects in telomerase repair genes have been linked to acquired aplastic anemia.[2]

Current therapeutic standards for AA include immunosuppressive treatment and allogeneic transplant, depending on disease severity, patient age and donor availability.[3-6] In the uncommon case of an identical twin, syngeneic transplant offers a rare therapeutic opportunity with significantly reduced treatment-related mortality. Furthermore, syngeneic transplantation has been performed with or without conditioning and with or without graft-*versus*-host disease (GvHD) prophylaxis, offering an insight into pathophysiology.

Along with numerous case reports, the largest series of syngeneic transplants in aplastic anemia reported on 37 and 40 patients, respectively, and were published over a decade ago.[7,8] Here, we present an analysis of all syngeneic transplants performed for aplastic anemia reported to the European Group for Blood and Marrow Transplantation (EBMT) registry.

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Methods

Study design

This study was conducted on behalf of the Severe Aplastic Anemia and Pediatric Diseases Working Parties of the EBMT. All EBMT centers report a minimal essential data set into a central database (MED-A Forms). After identification of eligible patients, missing information was requested from the individual centers. Informed consent was obtained locally according to the regulations applicable at the time of transplantation. Since January 1st 2003, the EBMT has required centers to confirm that written informed consent has been obtained prior to data collection. The EBMT is a collaborative group representing more than 500 transplantation centers in Europe.

Definitions

Severity at diagnosis was classified according to standard criteria.[3] Donor/recipient pairs were assumed to be syngeneic when reported as such by the transplant centers, and date of birth and sex were identical. For the purpose of this analysis, primary or secondary graft loss, reports of disease relapse as well as patients requiring a second transplant were classified as graft failure. Time to graft failure was defined as time between transplant and date of graft failure if provided, or date of subsequent transplant if the exact date of graft failure was not available. Only syngeneic transplants were included in the analysis. Selected patients received subsequent transplants from non-syngeneic donors after graft failure; these transplants were not included in the analysis and these patients were censored at the time of non-syngeneic transplant. Overall survival was defined as time from first transplantation until death. Transplant-related mortality was defined as death not related to relapse or graft failure.

Statistical analysis

Transplant characteristics were compared using Pearson's χ^2 test for categorical variables and Mann-Whitney U-test for continuous variables. Overall survival was estimated with the Kaplan-Meier method, while incidence of graft failure was calculated using the cumulative incidence method. The log rank test and Gray's test were used to compare among groups. Multivariable analysis was conducted using Cox models and stratified for transplant number. All covariates considered were forced into the model.

Results

Patients' characteristics

Between 1976 and 2009, 88 patients undergoing 113 syngeneic transplants were reported to the EBMT. Second, third and fourth syngeneic transplants were all performed with the same donor as the first. Median year of transplant was 1997. Median age at transplant was 21 years, with 33 patients being under 18 years of age at the time of transplant. Patient and transplant characteristics are shown in Tables 1 and 2.

Conditioning and posttransplant immunosuppression

Of the 88 patients, 65 received pre-transplant conditioning at first transplant whereas 18 did not (no information on 5 patients); including subsequent transplants, 85 transplants were preceded by conditioning. Information on type of conditioning was available in 71 patients; of these all but 5 received a cyclophosphamide-based regimen. Anti-thymocyte globulin (ATG) was given in 22 of 78 transplants, with data missing in 35. Thirty-nine patients received immunosuppressive treatment posttransplant while 47 did not; there were no data for the remaining 27 patients.

Transplant characteristics changed over time with 66% of transplants up to 1997 having been performed with conditioning, 96% with bone marrow, and 2% including ATG, *versus* 84% (P=0.013), 40% (P<0.001), and 37% (P<0.001), respectively, for transplants after 1997. No difference was seen for the administration of posttransplant immunosuppression.

Neutrophil engraftment

In the 74 transplants with data available, neutrophil

Table 1. Patients' characteristics. Y: years; M: male; F: female; d: days; AA: aplastic anemia; ATG: anti-thymocyte globulin.

	n=88
Median age at first transplant (range)	21y (2-69)
Gender	49 M, 39 F
Etiology	
Idiopathic	67
Toxic/post hepatic	10
Unknown	11
Severity at diagnosis	
Non-severe AA	5
Severe AA	18
Very severe AA	12
Unknown	53
Therapy before transplant	
ATG	6
Cyclosporine alone	3
Other treatment	29
No treatment	7
Unknown	43
Median time from diagnosis to first transplant (range)	84d (2-2141)

Table 2. Transplant characteristics by sequence. PBSC denotes peripheral blood stem cells, ATG anti-thymocyte globulin.

	All transplants n=113	1st transplant n=88	2nd transplant n=21	3rd transplant n=2	4th transplant n=2
Stem cell source					
Bone marrow	77	58	15	2	2
PBSC	36	30	6	0	0
Conditioning					
Yes	85	65	19	1	0
No	22	18	1	1	2
Unknown	6	5	1	0	0
Immunosuppression					
Yes	39	31	6	1	1
No	47	36	11	0	0
Unknown	27	21	4	1	1
ATG					
Yes	22	16	6	0	0
No	56	45	8	1	2
Unknown	35	27	7	1	0

engraftment (neutrophils >0.5 x 10^9/L) occurred after a median of 14 days (range 1-36). This was similar in patients with (13.5 days) and without conditioning (16.5 days) (P=0.379). Engraftment was more rapid after transplants with peripheral blood stem cells (PBSC) compared to bone marrow (median 12 days *vs.* 17 days; P=0.001).

Survival

With a median follow up of survivors of 7.28 years, the Kaplan-Meier estimate of 10-year (y) overall survival was 93% (Figure 1). Seven deaths occurred: 5 due to transplant-related mortality (3 after transplants with conditioning, 2 after transplants with no information on conditioning); and 2 of unknown causes. Causes of transplant-related mortality were: bleeding (n=1), infection (n=2), cardiac toxicity (n=1) and myelodysplastic syndrome (n=1). In total, 2 cases of secondary myelodysplastic syndrome were reported 7 and 16 months after transplant, both patients had received conditioning.

Graft failure

Figure 2 shows the outcome of transplants with and without conditioning. In transplants for which information on conditioning was missing (n=6), there were 2 cases of stable engraftment, 2 cases of graft failure, and 2 early deaths. Graft failure occurred in 36 of 113 (32%) transplants after a median of 332 days (range 22-3814). Graft failure occurred significantly more often after transplants without conditioning *versus* those with conditioning with 14 of 22 (64%) and 20 of 85 (24%), respectively (P<0.001).

Looking at the risk of graft failure after first transplant over time, the cumulative incidence (CI) at 3 years was 72% for transplants without conditioning *versus* 19% for those with conditioning (P<0.001) (Figure 3A). A further significant risk factor for transplant failure was graft source with a 3-year incidence of 37% versus 16% for bone marrow and PBSC, respectively (P=0.027) (Figure 3B). There was a trend towards increased risk of graft failure in transplants without posttransplant immunosuppression (3-year CI of 25% with and 42% without; P=0.282) (Figure 3C), while having received ATG in addition to conditioning had no influence (3-year CI of 30% and 27% with and without ATG, respectively, including only transplants with conditioning in the analysis; P=0.972) (Figure 3D). Further factors analyzed that had no impact on the risk of graft failure were age of the patient (below or above median), year of transplant (below or

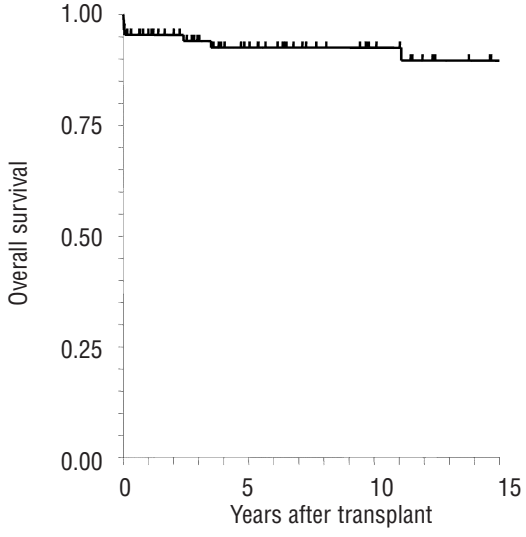

Figure 1. Overall survival.

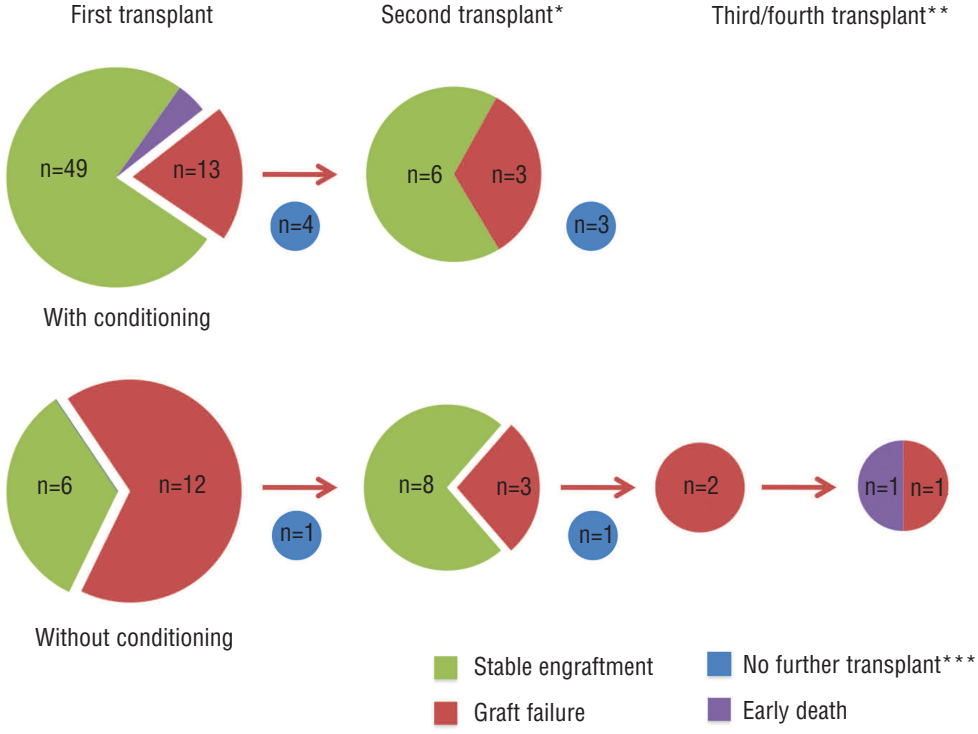

Figure 2. Outcome of first transplants with and without conditioning. *With conditioning in all but 1 patient. **1 of these was performed with conditioning, 3 without. ***No further transplant here refers specifically to syngeneic transplant for the treatment of aplastic anemia. Selected patients received further transplants from non-syngeneic donors that were not included in the analysis.

above median), and time from diagnosis to transplant (below or above median).

In a multivariate analysis of all transplants with stratification for transplant number including conditioning, posttransplant immunosuppression, graft source and year of transplant, only lack of conditioning retained a statistically significant influence on the risk of graft failure (HR 4.16; 95% confidence interval 1.88-9.20; $P<0.001$) (Table 3). However, there was also a trend for increased risk of graft failure with bone marrow *versus* PBSC, as well as in transplants without posttransplant immunosuppression.

Overall survival was not influenced by conditioning, graft source or posttransplant immunosuppression (*data not shown*).

Discussion

Here we describe a large cohort of syngeneic transplantation in aplastic anemia. Main findings include an excellent overall survival, as well as an increased risk of graft failure when transplanting without pre-transplant conditioning and with bone marrow as a stem cell source, and a trend towards improved engraftment with posttransplant immunosuppression. Factors without influence on risk of graft failure included ATG in patients with conditioning, as well as time from diagnosis to transplant, which could cautiously be interpreted as a potential surrogate marker for lack of influence of treatment before transplant or number of transfusions.

Numerous case reports have previously described patients who rejected a syngeneic graft without conditioning and engrafted successfully after a second transplant preceded by conditioning.[9-12] This finding was also confirmed in the CIBMTR cohort study of Hinterberger *et al.* in which all 13 patients who had received conditioning and survived more than 30 days had stable engraftment, while only 12 of 23 transplants without conditioning engrafted successfully.[7] A significant proportion of re-transplant after syngeneic transplant (38%) was also

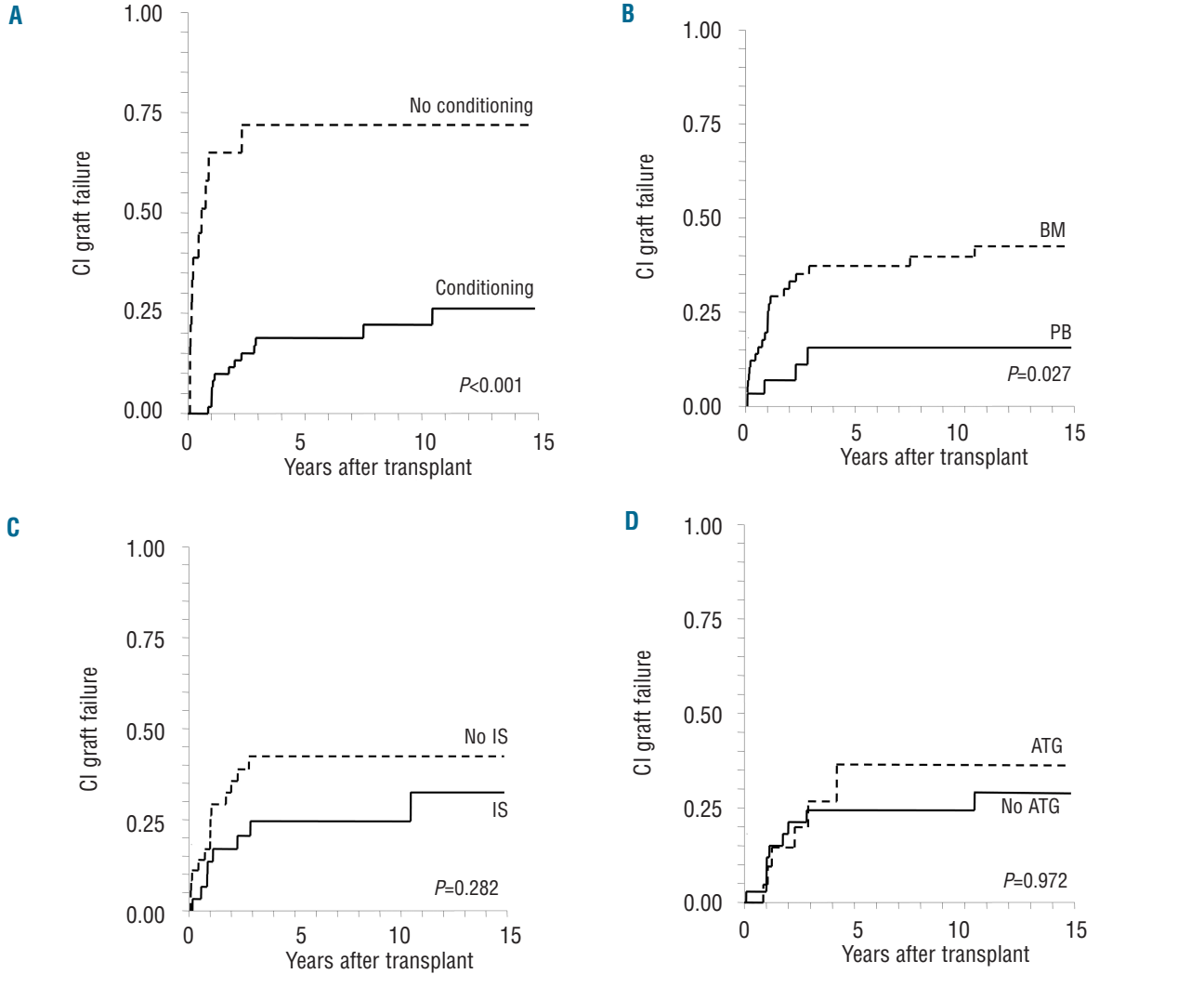

Figure 3. Cumulative incidence (CI) of graft failure with and without conditioning (A), with bone marrow (BM) or peripheral blood stem cells (PB) (B), with and without posttransplant immunosuppression (IS) (C), and with and without anti-thymocyte globulin (ATG) among transplants with conditioning (D). Only first transplants were analyzed.

reported by Bacigalupo *et al.*; however, no data on conditioning was provided.[8]

In both of these series, all patients received bone marrow as graft source. This has been associated with an increased risk of graft rejection compared to peripheral blood stem cells, especially following non-myeloablative conditioning.[13-15] However in our cohort, while bone marrow had a significant influence on the risk of graft failure in univariate analysis, peripheral stem cells could not overcome the significant risk of graft failure in transplants without conditioning, even when posttransplant immunosuppression was used. Nevertheless, it is interesting to note that there might be an advantage in using PBSC in syngeneic transplant due to the lack of risk of GvHD and potentially enhanced engraftment, since this is contrary to the current standard of using bone marrow in AA.[16]

The pathophysiology of AA has not been fully clarified. It is generally believed to be an autoimmune disease, although some patients may suffer from stem cell failure that is not attributable to a recognized congenital syndrome. In a patient with autoimmune disease, infusion of stem cells without pre-transplant conditioning is expected not to result in stable engraftment, whereas in patients with stem cell failure, conditioning might not be necessary if the donor is syngeneic. This was already inferred from case series several decades ago, where the necessity of conditioning for engraftment in most patients but not in all (as the minority had stable engraftment without pre-transplant conditioning) was thought to reflect the autoimmune pathogenesis in the majority of patients, while in a minority of cases the disease might be caused by a non-immune mechanism. This theory is further supported by the observation that the rate of graft failure in transplants without conditioning in our study (64%) and in the CIBMTR report (48%) is in line with the proportion of patients responding to ATG (60-70%).[1]

Some might argue that treatment success in selected cases of syngeneic transplant is, in fact, not due to the transplant but is only the result of the immunosuppressive conditioning and posttransplant immunosuppression; as we know, treatment of AA with high-dose cyclophosphamide has been a topic of debate in the past.[17,18] Though the inability to measure donor chimerism precludes proof of syngeneic engraftment, rapid neutrophil recovery, as well as lack of influence of ATG, are strong surrogate indicators of actual engraftment rather than response to immunosuppressive treatment, which usually occurs after several months.[3] Despite the widespread use of ATG, its role in pre-transplant conditioning is also not clear in the setting of matched sibling transplant, where a randomized study failed to confirm the positive effect on engraftment observed in a retrospective comparison.[19,20]

More recently, mutations in telomerase genes have been discovered in a subset of patients with apparently acquired aplastic anemia.[2,21] Patients with a mutation often showed no response to immunosuppressive treatment and, in some cases, the same mutation was found in family members with normal blood counts.[22,23] Fortunately, in our cohort, as well as in Hinterberger's,[7] almost all patients who rejected their first graft reached a complete remission following a second transplant preceded by conditioning, and we found no influence of conditioning on survival.

Table 3. Risk of graft failure. PBSC denotes peripheral blood stem cells.

	HR	95% confidence interval	P
Conditioning			
Yes	1.00		
No	4.16	1.88-9.20	<0.001
Immunosuppression			
Yes	1.00		
No	1.51	0.69-3.30	0.307
Graft source			
PBSC	1.00		
Bone marrow	1.68	0.58-4.82	0.337
Year of transplant			
Per year	1.00	0.95-1.05	0.993

However, a limited number of patients experienced repeated graft failure, and though we unfortunately lack information on telomere length in the patients and health of the syngeneic donors, selected cases might have been associated with unrecognized telomere gene mutations. Another possible explanation for repeated graft failure despite conditioning could be abnormal stromal microenvironment, as suggested in an earlier case report.[24] A further issue to consider is the fact that 2 patients developed myelodysplastic syndrome posttransplant, highlighting the importance of careful long-term follow up.

Worthy of note is the fact that no information was collected on how syngeneity of donor and recipients was established, or on how inherited bone marrow failure syndromes were excluded, particularly in the significant proportion of patients under 18 years of age. Hence, though we assume that centers took great care in confirming syngeneity and also in ruling out familial syndromes, we cannot completely exclude that selected donor/recipient pairs were not syngeneic or that selected patients suffered from an unidentified inherited syndrome, as discussed above.

Despite the further limitations of our study (its retrospective nature as well as missing data that could not be recovered since many of these transplants were performed over a decade ago), it is remarkable for the fact that it is the largest cohort of syngeneic transplants in aplastic anemia published to date, permitting statistical verification of previous observations. Based on these data we believe that pre-transplant conditioning, and possibly the preference to use peripheral blood as stem cell source, will be useful for successful syngeneic transplantation. Whether posttransplant immunosuppression or ATG are needed is less clear.

In summary, syngeneic transplant is a rare but precious treatment opportunity in aplastic anemia due to the excellent long-term survival and low transplant-related mortality. Moreover, it provides a unique opportunity to gain further insight into this rare disease, and outcome data should continue to be collected and analyzed regularly.

Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References

1. Young NS, Calado RT, Scheinberg P. Current concepts in the pathophysiology and treatment of aplastic anemia. Blood. 2006;108(8):2509-19.
2. Young NS, Bacigalupo A, Marsh JC. Aplastic anemia: pathophysiology and treatment. Biol Blood Marrow Transplant. 2010;16(1 Suppl):S119-25.
3. Marsh JC, Ball SE, Cavenagh J, Darbyshire P, Dokal I, Gordon-Smith EC, et al. Guidelines for the diagnosis and management of aplastic anaemia. Br J Haematol. 2009;147(1):43-70.
4. Scheinberg P, Young NS. How I treat acquired aplastic anemia. Blood. 2012; 120(6):1185-96.
5. Aljurf M, Al-Zahrani H, Van Lint MT, Passweg JR. Standard treatment of acquired SAA in adult patients 18-40 years old with an HLA-identical sibling donor. Bone Marrow Transplant. 2013;48(2):178-9.
6. Dufour C, Svahn J, Bacigalupo A, Severe Aplastic Anemia-Working Party of the E. Front-line immunosuppressive treatment of acquired aplastic anemia. Bone Marrow Transplant. 2013;48(2):174-7.
7. Hinterberger W, Rowlings PA, Hinterberger-Fischer M, Gibson J, Jacobsen N, Klein JP et al. Results of transplanting bone marrow from genetically identical twins into patients with aplastic anemia. Ann Intern Med. 1997;126(2):116-22.
8. Bacigalupo A, Oneto R, Bruno B, Socie G, Passweg J, Locasciulli A, et al. Current results of bone marrow transplantation in patients with acquired severe aplastic anemia. Report of the European Group for Blood and Marrow transplantation. On behalf of the Working Party on Severe Aplastic Anemia of the European Group for Blood and Marrow Transplantation. Acta Haematol. 2000;103(1):19-25.
9. Anderlini P, Riggs SA, Korbling M, Champlin R. Syngeneic blood stem cell transplantation for infectious mononucleosis-related aplastic anaemia. Br J Haematol. 1999;106(1):159-61.
10. Manley R, Fearnley D, Patton WN, Newhook C, Spearing RL, Hart DN. Syngeneic peripheral blood stem cell transplantation for severe aplastic anaemia. South Island Bone Marrow Transplant Team. Bone Marrow Transplant. 1997; 20(11):1009-10.
11. Appelbaum FR, Fefer A, Cheever MA, Sanders JE, Singer JW, Adamson JW, et al. Treatment of aplastic anemia by bone marrow transplantation in identical twins. Blood. 1980;55(6):1033-9.
12. Champlin RE, Feig SA, Sparkes RS, Galen RP. Bone marrow transplantation from identical twins in the treatment of aplastic anaemia: implication for the pathogenesis of the disease. Br J Haematol. 1984;56(3): 455-63.
13. Maris MB, Niederwieser D, Sandmaier BM, Storer B, Stuart M, Maloney D, et al. HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. Blood. 2003;102(6): 2021-30.
14. Anasetti C, Logan BR, Lee SJ, Waller EK, Weisdorf DJ, Wingard JR, et al. Peripheral-blood stem cells versus bone marrow from unrelated donors. N Engl J Med. 2012; 367(16):1487-96.
15. Baron F, Baker JE, Storb R, Gooley TA, Sandmaier BM, Maris MB, et al. Kinetics of engraftment in patients with hematologic malignancies given allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning. Blood. 2004;104(8): 2254-62.
16. Eapen M, Le Rademacher J, Antin JH, Champlin RE, Carreras J, Fay J, et al. Effect of stem cell source on outcomes after unrelated donor transplantation in severe aplastic anemia. Blood. 2011;118(9):2618-21.
17. Brodsky RA, Chen AR, Dorr D, Fuchs EJ, Huff CA, Luznik L, et al. High-dose cyclophosphamide for severe aplastic anemia: long-term follow-up. Blood. 2010; 115(11):2136-41.
18. Tisdale JF, Maciejewski JP, Nunez O, Rosenfeld SJ, Young NS. Late complications following treatment for severe aplastic anemia (SAA) with high-dose cyclophosphamide (Cy): follow-up of a randomized trial. Blood. 2002;100(13): 4668-70.
19. Champlin RE, Perez WS, Passweg JR, Klein JP, Camitta BM, Gluckman E, et al. Bone marrow transplantation for severe aplastic anemia: a randomized controlled study of conditioning regimens. Blood. 2007; 109(10):4582-5.
20. Storb R, Etzioni R, Anasetti C, Appelbaum FR, Buckner CD, Bensinger W, et al. Cyclophosphamide combined with antithymocyte globulin in preparation for allogeneic marrow transplants in patients with aplastic anemia. Blood. 1994;84(3): 941-9.
21. Calado RT, Young NS. Telomere diseases. N Engl J Med. 2009;361(24):2353-65.
22. Yamaguchi H, Calado RT, Ly H, Kajigaya S, Baerlocher GM, Chanock SJ, et al. Mutations in TERT, the gene for telomerase reverse transcriptase, in aplastic anemia. N Engl J Med. 2005;352(14):1413-24.
23. Fogarty PF, Yamaguchi H, Wiestner A, Baerlocher GM, Sloand E, Zeng WS, et al. Late presentation of dyskeratosis congenita as apparently acquired aplastic anaemia due to mutations in telomerase RNA. Lancet. 2003;362(9396):1628-30.
24. Marsh JC, Harhalakis N, Dowding C, Laffan M, Gordon-Smith EC, Hows JM. Recurrent graft failure following syngeneic bone marrow transplantation for aplastic anaemia. Bone Marrow Transplant. 1989; 4(5):581-5.