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Original Article

Not all anti-T lymphocyte globulin preparations are suitable for use in aplastic anemia: significantly inferior results with jurkat cell-reactive anti-T lymphocyte globulin in clinical practice

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Abstract: Background: Immunosuppressive therapy (IST) with anti-T lymphocyte globulin (ATG) plus cyclosporine (CSA) is standard therapy in patients with non-severe aplastic anemia (AA) in need of treatment and severe aplastic anemia (SAA) who do not have an available HLA-matched donor. The aim of this study was to analyze patients submitted to different ATG preparations as first-line treatment. Patients and methods: We retrospectively analyzed adult aplastic anemia (AA) patients who received ATG as first-line treatment between 1999 and 2013 to compare hematologic response and survival. Results: During the time period mentioned 4 different ATG preparations had been used in 38 AA patients (34 severe, 4 non-severe). Responses were better with Lymphoglobulin (6 complete response 1 partial response, 0 refractory disease and 2 death within 3 months after ATG, i.e. during induction), Thymoglobulin (3, 1, 4 and 1, respectively) or ATGAM (1, 2, 1 and 1) compared to the ATG-Fresenius (ATG-F) group (3, 0, 6 and 6) (P = .07). Statistically significant inferior results with ATG-Fresenius (3 complete or partial responses, 6 refractoriness and 6 induction deaths) were evident when other preparations are lumped together (14 complete or partial responses, 5 refractoriness and 4 induction mortalities) (P = .045). Estimated 1 year survival rates were 52.5% versus 76.9%, respectively (P = .13). Conclusions: These data support the notion that not all ATG preparations are suitable for use in AA.

Keywords: Aplastic anemia, immunosuppressive treatment, anti-T lymphocyte globulin

Introduction

Aplastic anemia (AA) is characterized by pancytopenia due to bone marrow aplasia. Although its pathogenesis has still not been fully understood, clinical observations and laboratory experiments have implicated an autoimmune mechanism. Autoimmunity leads to T cell activation and release of inhibitory cytokines to destroy hematopoietic stem and progenitor cells [1-3]. Bone marrow transplantation (BMT) and immunosuppressive treatment (IST) have improved outcome with remission rates of 60%-80% [4, 5]. However, shortages in the availability of the main IST agent for AA, anti-T lymphocyte globulin (ATG), have occurred during last decade. The combination of ATG and cyclosporine (CsA) is the gold standard immunosuppressive regimen for patients with severe aplastic anaemia and who do not have an HLA-matched donor for BMT, patients over 50 years of age, and patients with transfusion-dependent non-severe aplastic anaemia. HLA matched sibling BMT is used as first-line treatment for patients up to 50 years of age with SAA [6-8]. In our routine clinical practice the ATG preparation used for AA has repeatedly changed during last 1-2 decades due to drug shortages. This unpleasant condition gave us a possibility to compare successes of different ATG preparations in AA.

Patients and methods

We retrospectively analyzed adult patients with AA who received first-line therapy with ATG (± CsA) at Hacettepe University Hospital Department of Internal Medicine Section of Hema-

Table 1. Main baseline characteristics

	Horse (n = 14)		Rabbit (n = 24)		
	Lymphoglobulin (n: 9)	ATGAM (n: 5)	Thymoglobulin (n: 9)	ATG-F (n: 15)	P
	15 mg/kg 5 days	40 mg/kg 4 days	2.5-5 mg/kg 5 days	3-5 mg/kg 5-10 days	
Age, median (range)	44 (17-65)	43 (19-57)	36 (17-72)	40 (16-62)	.5
Gender (M/F)	7/2	1/4	5/4	5/10	.1
AA (severe/non-severe)	9/0	4/1	8/1	13/2	.64
Median f/u duration*	46.1 (14.7-135.9)	10.2 (6.1-19.6)	24.5 (2.7-133)	39.8 (11.8-90.4)	.32
Median f/u duration after CR/PR	12.1 (0-124)	2.9 (0-6.9)	33.4 (0-96.7)	39.4 (26.7-61.6)	.53
Diagnosis to ATG interval, median (range)	0.33 (0-4.5)	2.9 (0-3.3)	0.3 (0-41.1)	0.46 (0-21.6)	.7
Hemoglobin, median (range)	5.6 (2.4-9.2)	8.2 (5.6-11)	7.5 (6.5-12)	8.9 (3.7-12.3	.18
WBC count (range)	1800 (900-3400)	3000 (1500-3600)	2800 (1000-3400)	2100 (1200-4200)	.36
Platelet count, median (range)	44 (17-65)	43 (19-57)	36 (17-72)	40 (16-62)	.01**

*For surviving patients; **The difference is due to lower values in the Thymoglobulin group.

tology between 1999 and 2013. All patients who received at least one dose of ATG were included in the study. Surviving patients who had been followed for less than 3 months after ATG administration were not considered. Data were obtained from written and computerized medical records. Collected data included demographic information, pretreatment blood values, type of immunosuppressive therapy, date and number of courses of the IST, response to therapy, date of last known vital status for every patient. Baseline blood count values were defined as the lowest values within 4 weeks prior to the IST for elimination of transfusion and granulocyte colony-stimulating factor artifacts.

SAA was defined as a bone marrow cellularity of less than 25% with at least two of the following peripheral blood count criteria: (1) absolute neutrophil count (ANC) less than 0.5 × 109/L, (2) platelet count less than 20 × 109/L, and (3) corrected reticulocyte less than 1% [9]. Non-severe aplastic anemia was defined as pancytopenia not fulfilling the criteria for severe disease.

Treatment responses were classified as complete response (CR), partial response (PR), no response (NR) and induction mortality (for those who died without response within 3 months of ATG). CR was defined as transfusion independence associated with a hemoglobin concentration > 11 g/dL, neutrophil count > 1.5 × 109/L and a platelet count > 100 × 109/L. We defined PR as transfusion independence associated with a hemoglobin level > 8 g/dL, neutrophil count > 0.5 × 109/L, and a platelet count > 30 × 109/L. Other conditions including transfusion dependence were considered as no response. Exclusion criteria for this study were: (1) abnormal cytogenetics, (2) bone marrow findings consistent with myelodysplastic syndrome, (3) constitutional AA, and (4) diagnosis of paroxysmal nocturnal hemoglobinuria (PNH).

Statistical analyses

Categorical data were expressed as ratio and compared by the Chi-square (or Fisher's exact test if required by sample size). Continuous data were expressed as mean ± standard deviation or median (range) and compared by the Independent-samples T-test (or one-way ANOVA with Bonferroni post-hoc analysis if more than two parameters were compared). The primary outcomes were responses at 3 months and 1 year overall survival (OS). OS was calculated from date of ATG administration to the date of mortality of any reason by the Kaplan-Meier method. The patients still living at last follow up were censored at this time. Comparisons of survival rates were done by the Log-rank test. Statistical Packages for the Social Sciences v17.0 (SPSS Inc., Chicago, IL) software was used for statistical analyses. A p value < 0.05 was considered to be significant.

Results

Descriptive patient data according to treatment groups

38 patients (34 severe and 4 non-severe AA) were included in the study. The median age at diagnosis was 39 years, with a range of 16 to 72 years. There was a nearly equal gender distribution with 18 male and 20 female patients.

Table 2. ATG preparations that were used for first line treatment year by year from 1999 through 2013

Sum	2	3	1	2	6	6	4	3	2	1	3	1	2	2	38
L	2	1	0	1	2	2	1	0	0	0	0	0	0	0	9
T	0	2	1	1	4	0	1	0	0	0	0	0	0	0	9
F	0	0	0	0	0	4	2	3	2	1	3	0	0	0	15
A	0	0	0	0	0	0	0	0	0	0	0	1	2	2	5
	1999	2000	2001	2002	2003	2004	2005	2006	2008	2009	2010	2011	2012	2013	2014

L: Lymphoglobulin, T: Thymoglobulin, F: ATG-Fresenius, A: ATGAM.

Table 3. Treatment response in different ATG preparations

	Horse		Rabbit		P
	Lymphoglobulin	ATGAM	Thymoglobulin	ATG-F	
Treatment response in 4 groups (CR/PR/NR/induction mortality)	6/1/0/2	1/2/1/1	3/1/4/1	3/0/6/6	.07
Treatment response ATG-F vs others (CR or PR/NR/induction mortality)	14/5/4			3/6/6	.045
Relapsing patients/CR+PR	0/7	0/3	0/4	0/3	
Overall survival at one year in 4 groups	77.8	80	74.1	52.5	.52
Overall survival at one year (ATG-F vs others)		76.9		52.5	.13

Both Lymphoglobulin (Genzyme, Cambridge, MA, USA) and Thymoglobulin (Genzyme, Cambridge, MA, USA) could be used until 2005 (**Table 2**). Only ATG-F (Fresenius Biotech GmbH, Germany) was available from 2006 to 2010. ATGAM (Pfizer, Kalamazoo, MI, USA) could be used from 2011 on. These preparations were used as first line treatment in 9, 9, 15, and 5 patients, respectively. Important baseline descriptive data of the patients according to treatment groups are presented in **Table 1**. The treatment groups were statistically similar for all baseline parameters except for a lower baseline platelet count in the Thymoglobulin group.

Treatment responses and survival

17 responses (13 complete and 4 partial), 11 refractory disease and 10 induction mortalities were observed. Distributions of the responses and overall survival in the treatment groups are presented in **Table 3**. Responses were significantly better in other groups lumped together (14 CR or PR, 5 NR and 4 induction mortalities) compared to the ATG-F group (3, 6 and 6, respectively) (P = 0.045). Two of the 3 patients responding to ATG-F were non-severe AA. Estimated 1 year survival rates were 76.9% versus 52.5%, respectively (P = .13, **Figure 1**). 4 out of 11 non-responding (and surviving beyond 3 months after ATG) patients received secondary treatments including 1 allogeneic transplant and 3 alternative ATG preparations (2 Thymoglobulin and 1 ATGAM). After a median follow-up duration of 14.2 months (3-131) 2 of 11 died and one patient developed myelodysplastic syndrome. All of the 17 responding patients were surviving after a median of 42 months (3-131) after ATG. No relapse or secondary hematopoietic disorders have occurred in the responding patients.

Discussion

Despite biologic similarities in manufacturing of various ATG preparations, there are many differences in pharmacokinetics and in effects on immune system. These differences may change their efficacy in restoring hematopoiesis in AA patients. Generally, studies on successes of different ATG preparations have focused on the difference between horse and rabbit products.

A few investigators have recently compared the efficacy of IST with horse ATG (h-ATG) versus rabbit ATG (r-ATG) in AA patients [10-21]. The majority of the published studies comparing r-ATG to h-ATG to date have included relatively small numbers of patients and the results have been generally conflicting. Some studies have demonstrated similar response rates to r-ATG and h-ATG [10-14]. Other studies indicated significantly worse response rates and survival for AA patients treated with r-ATG compared to h-ATG [15, 16].

In an US prospective study, a significantly lower percentage of patients treated with r-ATG achieved a response to treatment compared to

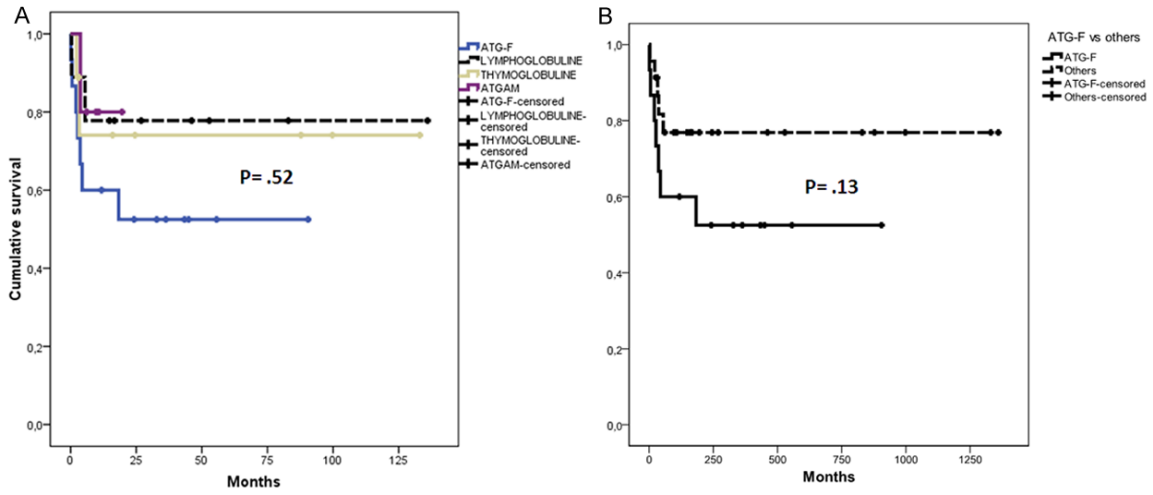

Figure 1. A. Overall survival in the 4 treatment groups. B. Overall survival in the ATG-F group and all others lumped together.

those treated with h-ATG (P < 0.001) [15]. In a recently conducted prospective study by the European Blood and Marrow Transplant Group, the patients who received IST with h-ATG also showed superior OS in comparison with r-ATG (86 versus 68 %, P = 0.009) in spite of acceptable response rates in both groups (67 and 60 %, respectively) [16]. Although there are conflicting reports it should be noticed that prospective randomized studies have indicated that h-ATG was superior to r-ATG.

Traditionally, h-ATG is the preferred ATG preparation for patients with AA. For some time, however, r-ATG was the only ATG formulation available due to difficulties in manufacturing h-ATG in many countries. In Turkey, centers had to use ATG-F to treat aplastic anemia, because no other product was available for a while. In two recent studies from Turkey during this period, patients with SAA receiving jurkat-cell reactive rabbit ATG (ATG-F) as a first-line treatment did not show acceptable response rates [17, 18]. One of those studies [17] by our group considered the patients who were treated between 1993 and 2004. That study was one of the first studies reporting worse results with an r-ATG product, namely ATG-F. The current study which includes the patients who were treated between 1999 and 2004 in common with our previous report confirms our previous results in a larger cohort. We preferred to limit this study to last 15 years in order to minimize the impact of better modern supportive care modalities on treatment results.

It is important to bear in mind that all of the studies which have reported good response rates with an r-ATG used Thymoglobulin. In this study we observed CR (3) or PR (1) in 4 out of 9 Thymoglobulin patients. In ATG-F group only 3 patients had a response (CR), and it should be pointed out that 2 of them were in non-severe group. In fact ATG-F is not an anti-T lymphocyte or anti-thymocyte globulin. It is isolated from the serum of rabbits immunized with the human Jurkat cell line of T lymphoblasts instead of thoracic duct lymphocytes or thymocytes. Although limited, there are data indicating that these preparations have different mechanisms of action. The therapeutic mechanisms of the classical agents are immunosuppressive, immunostimulatory and direct effects on hematopoietic stem cells. However, the mechanism of ATG-F is restricted to an immunosuppressive effect [19]. Non-severe aplastic anemia patients have a hematopoietic stem cell reserve, so immunosuppressive effect of ATG-F could be sufficient for a response.

Regarding other studies reporting about ATG-F in AA, the majority of them reported similar unfavourable results [20, 21]. However, one pediatric study found that ATG-F and r-ATG had similar efficacy and adverse reactions in the first-line treatment of childhood AA [22].

This study has several limitations. The study was retrospective and based on a relatively small number of patients. The low patient numbers were due to the fact that immunosuppres-

sive treatment has only been indicated for cases not eligible for allogeneic transplantation.

In conclusion, these data support the notion that not all ATG preparations are suitable for use in AA. ATG-F seems to be not adequate for treatment of SAA at least in the described dosage.

Disclosure of conflict of interest

None.

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References

[1] Marsh JC, Ball SE, Cavenagh J, Darbyshire P, Dokal I, Gordon-Smith EC, Keidan J, Laurie A, Martin A, Mercieca J, Killick SB, Stewart R, Yin JA; British Committee for Standards in Haematology. Guidelines for the diagnosis and management of aplastic anaemia. Br J Haematol 2009; 147: 43-70.

[2] Young NS, Calado RT, Scheinberg P. Current concepts in the pathophysiology and treatment of aplastic anemia. Blood 2006; 108: 2509-2519.

[3] Bacigalupo A. Aplastic anemia: pathogenesis and treatment. Hematol Am Soc Hematol Educ Program 2007; 23-8.

[4] Wingard JR, Majhail NS, Brazauskas R, Wang Z, Sobocinski KA, Jacobsohn D, Sorror ML, Horowitz MM, Bolwell B, Rizzo JD, Socié G. Long-term survival and late deaths after allogeneic hematopoietic cell transplantation. J Clin Oncol 2011; 29: 2230-2239.

[5] Locasciulli A, Oneto R, Bacigalupo A, Socié G, Korthof E, Bekassy A, Schrezenmeier H, Passweg J, Führer M; Severe Aplastic Anemia Working Party of the European Blood and Marrow Transplant Group. Outcome of patients with acquired aplastic anemia given first line bone marrow transplantation or immunosuppressive treatment in the last decade: a report from the European Group for Blood and Marrow Transplantation (EBMT). Haematologica 2007; 92: 11-18.

[6] Di Bona E, Rodeghiero F, Bruno B, Gabbas A, Foa P, Locasciulli A, Rosanelli C, Camba L, Saracco P, Lippi A, Iori AP, Porta F, De Rossi G, Comotti B, Iacopino P, Dufour C, Bacigalupo A. Rabbit antithymocyte globulin (r-ATG) plus cyclosporine and granulocyte colony stimulating factor is an effective treatmentfor aplastic anaemia patients unresponsive to a first courseof intensive immunosuppressive therapy. Gruppo Italiano Trapianto diMidollo Osseo (GITMO). Br J Haematol 1997; 107: 330-334.

[7] Marsh JC, Ball SE, Cavenagh J, Darbyshire P, Dokal I, Gordon-Smith EC, Keidan J, Laurie A, Martin A, Mercieca J, Killick SB, Stewart R, Yin JA; British Committee for Standards in Haematology. Guidelines for the management of aplastic anaemia. Br J Haematol 2009; 147: 43-70.

[8] Scheinberg P, Young NS. How I treat acquired aplastic anemia. Blood 2012; 120: 1185-96.

[9] Bacigalupo A, Bruno B, Saracco P, Di Bona E, Locasciulli A, Locatelli F, Gabbas A, Dufour C, Arcese W, Testi G, Broccia G, Carotenuto M, Coser P, Barbui T, Leoni P, Ferster A. Antilymphocyte globulin, cyclosporine, prednisolone, and granulocyte colony-stimulating factor for severe aplastic anemia: an update of the GITMO/EBMT study on 100 patients. European Group for Blood and Marrow Transplantation (EBMT) Working Party on Severe Aplastic Anemia and the Gruppo Italiano Trapianti di Midolio Osseo (GITMO). Blood 2000; 95: 1931-4.

[10] Shin SH, Yoon JH, Yahng SA, Lee SE, Cho BS, Eom KS, Kim YJ, Lee S, Min CK, Kim HJ, Cho SG, Kim DW, Min WS, Park CW, Lee JW. The efficacy of rabbit antithymocyte globulin with cyclosporine in comparison to horse antithymocyte globulin as a first-line treatment in adult patients with severe aplastic anemia: a single-center retrospective study. Ann Hematol 2013; 92: 817-824.

[11] Afable MG II, Shaik M, Sugimoto M, Elson P, Clemente M, Makishima H, Sekeres MA, Lichtin A, Advani A, Kalaycio M, Tiu RV, O'Keefe CL, Maciejewski JP. Efficacy of rabbit antithymocyte globulin in severe aplastic anemia. Haematologica 2011; 96: 1269-1275.

[12] Vallejo C, Montesinos P, Polo M, Cuevas B, Morado M, Rosell A, Xicoy B, Díez JL, Salamero O, Cedillo Á, Martínez P, Rayón C; Bone Marrow Failure Spanish Study Group (Pethema-GETH). Rabbit antithymocyte globulin versus horse antithymocyte globulin for treatment of acquired aplastic anemia: a retrospective analysis. Ann Hematol 2015; 94: 947-54.

[13] Chang MH, Kim KH, Kim HS, Jun HJ, Kim DH, Jang JH, Kim K, Jung CW. Predictors of response to immunosuppressive therapy with antithymocyte globulin and cyclosporine and prognostic factors for survival in patients with severe aplastic anemia. Eur J Haematol 2010; 84: 154-159.

[14] Sakamoto T, Obara N, Kurita N, Sakata-Yanagimoto M, Nishikii H, Yokoyama Y, Suzukawa K, Hasegawa Y, Chiba S. Effectiveness and safety of rabbit anti-thymocyte globu-

lin in Japanese patients with aplastic anemia. Int J Hematol 2013; 98: 319-322.
[15] Scheinberg P, Nunez O, Weinstein B, Scheinberg P, Biancotto A, Wu CO, Young NS. Horse versus rabbit antithymocyte globulin in acquired aplastic anemia. N Engl J Med 2011; 365: 430-438.
[16] Marsh JC, Bacigalupo A, Schrezenmeier H, Tichelli A, Risitano AM, Passweg JR, Killick SB, Warren AJ, Foukaneli T, Aljurf M, Al-Zahrani HA, Höchsmann B, Schafhausen P, Roth A, Franzke A, Brummendorf TH, Dufour C, Oneto R, Sedgwick P, Barrois A, Kordasti S, Elebute MO, Mufti GJ, Socie G; European Blood and Marrow Transplant Group Severe Aplastic Anaemia Working Party. Prospective study of rabbit antithymocyte globulin and cyclosporine for aplastic anemia fromthe EBMT Severe Aplastic Anaemia Working Party. Blood 2012; 119: 5391-5396.
[17] Serefhanoglu S, Buyukasik Y, Purnak T, Goker H, Sayinalp N, Haznedaroglu IC, Ozcebe OI. A comparison of Jurkat cell-reactive anti-T lymphocyte globulin and fetal anti-thymocyte globulin preparations in the treatment of aplastic anemia. Med Princ Pract 2011; 20: 341-344.
[18] Karadaş N, Ay Y, Akin M, Balkan C, Aydinok Y, Kavakli K. Rabbit antithymocyte globulin treatment in childhood acquired severe aplastic anemia. Karapinar DY 2014; 31: 20-8.
[19] Eiermann TH, Freitag S, Cortes-Dericks L, Sahm H, Zander AR. Jurcat cell-reactive antithymocyte globulin assessed ex vivo by flow cytometry persists three weeks in circulation. J Hematother Stem Cell Res 2001; 10: 385-390.
[20] Shao YQ, Li XX, Ge ML, Shi J, Zhang J, Huang JB, Huang ZD, Nie N, Zheng YZ. A long-term follow up study on 345 severe aplastic anemia patients treated with antithymocyglobulin/lymphoglobulin. Zhonghua Xue Ye Xue Za Zhi 2013; 34: 30-5.
[21] Zheng Y, Liu Y, Chu Y. Immunosuppressive therapy for acquired severe aplastic anemia (SAA): a prospective comparison of four different regimens. Exp Hematol 2006; 34: 826-31.
[22] Xie X, Shi W, Zhou X, Shao Y, Qiao X. Comparison of rabbit antithymocyte globulin and Jurkat cell-reactive anti-T lymphocyte globulin as a first-line treatment for children with aplastic anemia. Exp Hematol 2014; 42: 431-8.