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 mortalities) 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-
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Table 1. Main baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Horse (n = 14)</th>
<th>Rabbit (n = 24)</th>
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<tbody>
<tr>
<td></td>
<td>Lymphoglobulin (n: 9)</td>
<td>ATGAM (n: 5)</td>
<td>Thymoglobulin (n: 9)</td>
</tr>
<tr>
<td>Age, median (range)</td>
<td>44 (17-65)</td>
<td>43 (19-57)</td>
<td>36 (17-72)</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>7/2</td>
<td>1/4</td>
<td>5/4</td>
</tr>
<tr>
<td>AA (severe/non-severe)</td>
<td>9/0</td>
<td>4/1</td>
<td>8/1</td>
</tr>
<tr>
<td>Median f/u duration*</td>
<td>46.1 (14.7-135.9)</td>
<td>10.2 (6.1-19.6)</td>
<td>24.5 (2.7-133)</td>
</tr>
<tr>
<td>Median f/u duration after CR/PR</td>
<td>12.1 (0-124)</td>
<td>2.9 (0-6.9)</td>
<td>33.4 (0-96.7)</td>
</tr>
<tr>
<td>Diagnosis to ATG interval, median (range)</td>
<td>0.33 (0-4.5)</td>
<td>2.9 (0-3.3)</td>
<td>0.3 (0-4.1)</td>
</tr>
<tr>
<td>Hemoglobin, median (range)</td>
<td>5.6 (2.4-9.2)</td>
<td>8.2 (5.6-11)</td>
<td>7.5 (6.5-12)</td>
</tr>
<tr>
<td>WBC count (range)</td>
<td>1800 (900-3400)</td>
<td>3000 (1500-3600)</td>
<td>2800 (1000-3400)</td>
</tr>
<tr>
<td>Platelet count, median (range)</td>
<td>44 (17-65)</td>
<td>43 (19-57)</td>
<td>36 (17-72)</td>
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</table>

*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 x 10⁹/L, (2) platelet count less than 20 x 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 x 10⁹/L and a platelet count > 100 x 10⁹/L. We defined PR as transfusion independence associated with a hemoglobin level > 8 g/dL, neutrophil count > 0.5 x 10⁹/L, and a platelet count > 30 x 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.
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Table 2. ATG preparations that were used for first line treatment year by year from 1999 through 2013

<table>
<thead>
<tr>
<th>Year</th>
<th>L</th>
<th>T</th>
<th>F</th>
<th>A</th>
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<tbody>
<tr>
<td>1999</td>
<td>2</td>
<td>1</td>
<td>0</td>
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<td>2002</td>
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<td>4</td>
<td>0</td>
<td>3</td>
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<td>2003</td>
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<tr>
<td>2014</td>
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</table>

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

Table 3. Treatment response in different ATG preparations

<table>
<thead>
<tr>
<th>Horse</th>
<th>Rabbit</th>
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</thead>
<tbody>
<tr>
<td>Lymphoglobulin</td>
<td>ATGAM</td>
<td>Thymoglobulin</td>
</tr>
<tr>
<td>Treatment response in 4 groups (CR/PR/NR/induction mortality)</td>
<td>6/1/0/2</td>
<td>1/2/1/1</td>
</tr>
<tr>
<td>Treatment response ATG-F vs others (CR or PR/NR/induction mortality)</td>
<td>14/5/4</td>
<td>3/6/6</td>
</tr>
<tr>
<td>Relapsing patients/CR+PR</td>
<td>0/7</td>
<td>0/3</td>
</tr>
<tr>
<td>Overall survival at one year in 4 groups</td>
<td>77.8</td>
<td>80</td>
</tr>
<tr>
<td>Overall survival at one year (ATG-F vs others)</td>
<td>76.9</td>
<td>52.5</td>
</tr>
</tbody>
</table>

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
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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 transplanta-
tion.

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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