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ORIGINAL ARTICLE

Antithymocyte Globuline Therapy and Bradycardia in Children

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Abstract

In antithymocyte globulin (ATG) treated patients occasionally bradycardia has been noticed. Therefore, we retrospectively analyzed the occurrence of bradycardia in ATG-treated children. Using medical records between 2007 and 2012 we identified children undergoing a combined therapy with ATG and glucocorticoids (ATG group, $n = 22$). The incidence of bradycardia was compared to that registered in children treated with glucocorticoids alone (glucocorticoid alone group, $n = 21$). Heart rates (HR) were registered before and on days 0–3, 4–7 and 8–14 after the ATG or steroid administration. The rate of bradycardic episodes was higher during ATG therapy than in the steroid alone group, while severe bradycardia occurred only in the ATG group (97 versus 32, $p = 0.0037$, and 13 versus 0, $p = 0.0029$, respectively). There was an interaction between the time and treatment group on HR ($p = 0.046$). Heart rates in ATG and steroid alone groups differed significantly on day 0–3 and day 4–7 ($p = 0.046$, $p = 0.006$, respectively). Within the ATG group HR was lower on days 4–7 compared to the days before and the days 8–14 values ($p < 0.001$, 95%CI: 0.020–0.074). These findings indicate that transient asymptomatic bradycardia is probably more common with ATG therapy than previously reported. HR should be closely monitored during and after ATG therapy.

Keywords Antithymocyte globulin · ATG · Bradycardia · Children

Introduction

Antithymocyte globulin (ATG) is an immunosuppressive agent used in pediatric hematology for decades in two major indications. It is given to prevent the development of graft versus host disease (GvHD) after hematopoietic stem cell transplantation (HSCT) [1]. In addition, it is the first-line therapy recommended for severe aplastic anemia in the absence of a sibling donor [2–4]. ATG products currently approved for human use are polyclonal rabbit (ATG Fresenius, Neovii Biotech Gmbh., Munich, Germany; Thymoglobin, Genzyme Corporation, Cambridge, Massachusetts, USA) or horse (ATGAM, Pfizer Corporation, New York, NY, USA) antibodies against human T-cells.

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The primary action of ATG is the eradication of certain T-cell clones. ATG recognizes several markers on the cell surface (CD2, CD3, CD4, CD8, CD11a, CD18, CD25, HLA-DR, etc.), most of them playing an important role in T-cell activation cascade. T-cells are eliminated from the blood by complement-mediated lysis and Fc-mediated opsonization and phagocytosis. Elimination half-life time varies between 3 and 14 days. The majority of T-cell subgroups repopulate after 2 months, but the absolute CD4+ helper lymphocyte count can be seriously depressed for up to 6 months. In the HSCT setting T cell recovery in the host is dependent of other transplant related factors as well. ATG does not have a clear effect on B-cells.

Recent guidelines recommend the use of the horse ATG due to improved efficacy in severe aplastic anemia compared to that of rabbit ATG [5, 6]. On the contrary, most transplantation protocols refer to rabbit ATG for GvHD prophylaxis. These agents have been in use for more than 3 decades; therefore, their side effect profile is thought to be well defined. Major adverse events during ATG therapy are lymphopenia, leukopenia, thrombocytopenia, allergic reactions, serum sickness and infections. In 2007, we unexpectedly observed a sudden decrease of the heart rate after the administration of

ATG in several HSCT patients with normal baseline cardiac function. This observation was supported by a more recent case report in 2011 describing the worsening of bradycardia [7]. These observations initiated a retrospective data analysis of heart rate characteristics during and early after ATG therapy in our patients.

Materials and Methods

Patients Between 2007 and 2012 severe aplastic anemia was treated with ATG in 12 pediatric cases. Ten other patients were transplanted in 2012 with a matched unrelated donor, for whom ATG was given as GvHD prophylaxis. ATG group consisted of these 12 patients treated with SAA and 10 patients with GvHD prophylaxis (ATG patients; $n = 22$).

In accordance with our institutional guidelines all ATG patients were given corticosteroid (methylprednisolone, 1-4 mg/kg/day, intravenously) for the prevention of serious allergic reactions and serum sickness. As bradycardia has been reported as a possible side effect of glucocorticoids [8–11], the ATG group was compared to a group of patients with GvHD treated with high dose steroids alone (methylprednisolone, 2-5 mg/kg/day, intravenously) (steroid alone group, $n = 21$). Patient characteristics are shown in Table 1.

Drugs Horse ATG (ATGAM) was used only for the treatment of SAA at a dose of 40 mg/kg/day for 4 consecutive days, administered intravenously ($n = 3$ patients). Rabbit ATG (Thymoglobin), was administered at 5 mg/kg/day ($n = 16$), intravenously for 4 consecutive days for SAA treatment ($n = 7$), and 2.5 mg/kg/day for 4 consecutive days for GvHD prophylaxis ($n = 9$). The dose of the other rabbit ATG (ATG Fresenius) was 15 mg/kg/day ($n = 3$), intravenously for 7 consecutive days for SAA therapy ($n = 2$) and for 3 consecutive days for GvHD prophylaxis ($n = 1$). Each patient received high-dose corticosteroid (methylprednisolone, 1-4 mg/kg/day, intravenously) for the prevention of allergic adverse reactions and serum sickness from day 1 of the ATG treatment for up to three weeks daily. The patients in the steroid alone group received high dose corticosteroid (methylprednisolone, 2-5 mg/kg/day, intravenously) for de novo acute GvHD for at least a month. This regime was strictly followed in all cases. As a part of the routine investigation according to the institutional protocol a thorough cardiologic examination with cardiac ultrasonography and ECG was performed in all patients before the transplantation. Concomitant administration of drugs with reported side effects on cardiac function should have render patients ineligible for the survey; however, no individual fulfilled these exclusion criteria.

Measurement Routine observational protocol was carried out during the treatment period. Heart rate, blood pressure, body temperature, respiratory rate and oxygen saturation with pulse oximetry were recorded in every 4 h. Vital parameters were recorded before the therapy (pretreatment), on days 0–3, 4–7 and 8–14 after the first day of the ATG and steroid administration. A total of 5013 vital signs were registered and analyzed. A complete blood count with detailed chemistry including electrolytes was obtained daily. Venous blood gas parameters and serum lactate was measured at bedside when necessary, but at least once a day. Electrocardiography (ECG) was not performed routinely when patients had no cardiac complaints and were clinically and hemodynamically stable. Our institutional protocol required to maintain an adequate hemoglobin level, hydration, electrolyte and oxygen status during observation so these cofounding factors did not influence the heart rate. None of the patients had other concomitant illness, such as thyroid problem as well.

Bradycardia Bradycardia was defined as a heart rate lower than 100/min at the age of 0–2 years, lower than 60/min at the age of 2–9 years and lower than 50/min above 9 years of age was [12], while a heart rate lower than 60/min at the age of 0–5 years, lower than 45/min at the age of 6–11 years and lower than 40/min above 11 years of age was specified as severe bradycardia [13].

Statistical Analysis For the statistical analyses, we used the IBM SPSS Statistics 20 program (New York, NY, USA). A mixed analysis of variance (ANOVA) procedure was used to determine whether measured heart rates over time differ between the two treatment groups. We also tested the interaction between the two independent variables (i.e. ATG and steroid therapy) and to estimate effect sizes on bradycardia. The observed heart rates were logarithmically transformed to get normal distribution. There was homogeneity of variances and covariances, as assessed by Levene's test of homogeneity of variance ($p > 0.05$) and Box's test of equality of covariances matrices ($p > 0.001$). We determined the differences between groups at each time point and vice versa by simple main effects methods using univariate ANOVA (testing for the simple main effects for the treatment groups) and repeated measures ANOVA (testing for the simple main effects for time). The p-values were corrected for multiple comparisons using the Bonferroni correction. The frequency of bradycardic and severe bradycardic episodes in ATG and steroid alone groups were compared with Chi-squared and Fisher's exact tests. The level of significance was set at p-values less than 0.05.

Results

The observed heart rates at the different time points after the two treatment groups (ATG vs. steroid) are shown on Fig. 1. The logarithmic transformed values of the heart rates are

Table 1 Patients characteristics

		All	ATG group	Steroid alone group
Patients		43	22	21
	Male	25	14	11
	Female	18	11	7
	Median age (years)	10.8 (0.5–17.7)	10.97 (0.92–17.73)	10.52 (0.53–16.61)
Diagnosis	SAA	17	15	2
	ALL	8	0	8
	AML	5	1	4
	CML	1	0	1
	MDS	1	1	0
	NHL	2	1	1
	NBL	1	1	0
	Inborn error	5	1	4
	Autoimmune diseases	3	2	1
Groups	ATG therapy	12	12	0
	ATG prophylaxis	10	10	0
	Steroid	21	0	21
ATG type	Horse	3	3	0
	Rabbit	19	19	0
Product	ATGAM	3	3	0
	ATG Fresenius	3	3	0
	Thymoglobin	16	16	0

shown on Fig. 2. Detailed information regarding the descriptive statistics of the heart rate values is shown in Table 2. The incidence of bradycardic episodes was higher in the ATG than in the steroid alone group ($p = 0.0039$, Table 3), and severe bradycardia was observed only in the ATG group ($p = 0.0029$, Table 4). There was an interaction between time and treatment group on heart rate ($F = 2.750$, $p = 0.046$, partial $\eta^2 = 0.066$). There was a difference in heart rates of ATG and steroid alone

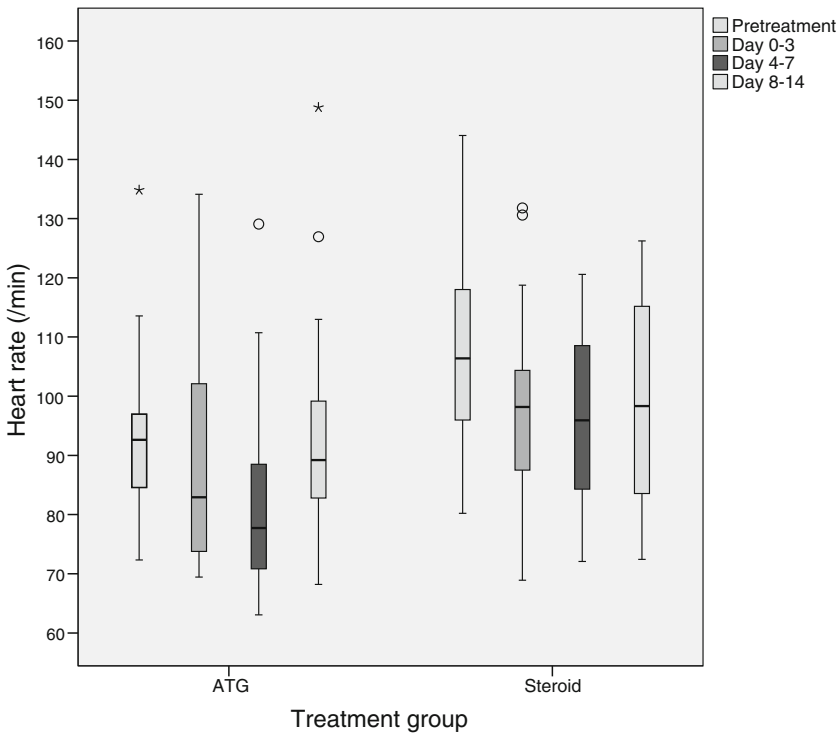

Fig. 1 The box plots represent the heart rate (/min) measured before the treatments and on days 0–3, 4–7 and 14 after the ATG and steroid treatments. The top of the box plot represents the 75th percentile, the bottom of the box plot represents the 25th percentile, and the line in the middle represents the median value of values recorded during the investigated time period. The whiskers represent the highest and lowest values that are not outliers or extreme outliers. Circular dots represent outlier data points that are more than 1.5 box-lengths from the edge of their box. Asterisks represent extreme outlier data points that are more than 3 box-lengths away from the edge of their box

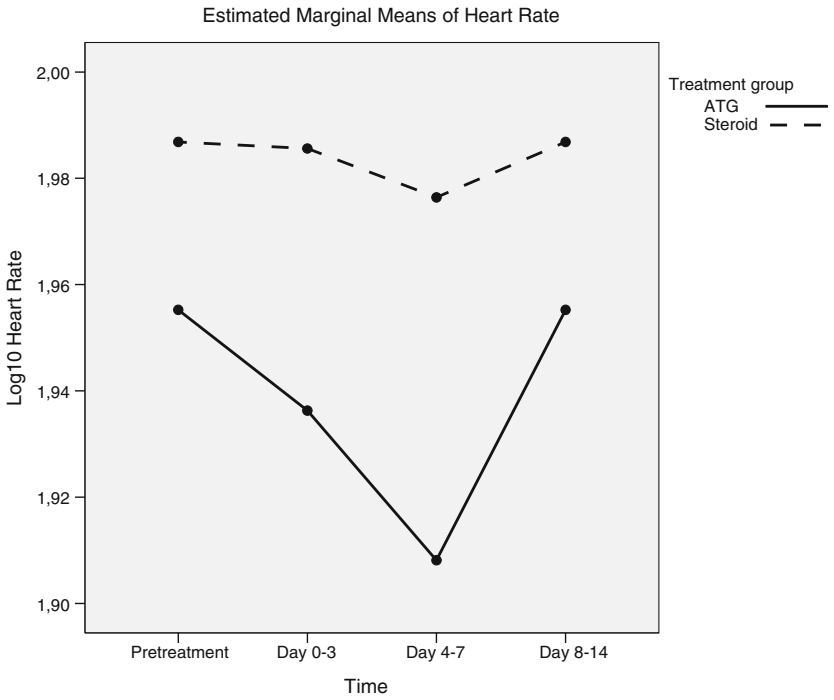

Fig. 2 There was difference in heart rates between the ATG and steroid alone groups on days 0–3 (F = 4.388, $p = 0.046$, partial $\eta^2 = 0.101$) and days 4–7 (F = 8.518, $p = 0.006$, partial $\eta^2 = 0.179$). Heart rate was lower on days 4–7 compared to the pretreatment and days 8–14 values (M = 0.047, 95%CI: 0.020–0.074, $p < 0.001$, separately) in the ATG group

groups on days 0–3 (F = 4.388, p = 0.046, partial $\eta^2 = 0.101$) and days 4–7 (F = 8.518, $p = 0.006$, partial $\eta^2 = 0.179$). There was an effect of time on heart rate for the ATG group (F = 8.09, $p < 0.001$, partial $\eta^2 = 0.278$). The heart rate of the ATG group was lower on days 4–7 compared to the pretreatment and days 8–14 values (M = 0.047, 95%CI: 0.020–0.074, p < 0.001, separately). Age, sex, other vital parameters, laboratory results, comorbidities, ATG types (horse or rabbit, manufacturer) or indications (prophylaxis or therapy) were comparable in patients with and without bradycardia. Low heart rate was due to sinus bradycardia in all cases documented by ECG without any clinical consequences.

Table 2 Descriptive statistics of the heart rate values

Group	Statistics		Pretreatment	Day_0_3	Day_4_7	Day_8_14
ATG	Mean		94.31	87.76	82.25	91.82
	Standard error of mean		2.83	3.62	3.42	4.01
	95% Confidence interval for mean	Lower limit	88.43	80.23	75.13	83.47
		Upper limit	100.19	95.28	89.37	100.16
	Median		92.63	82.93	77.74	89.21
	Variance		175.98	287.90	257.84	354.11
	Std. deviation		13.27	16.97	16.06	18.82
	Minimum		72.33	69.45	63.05	68.20
	Maximum		134.81	134.11	129.08	148.78
Steroid	Mean		107.59	98.01	95.92	98.53
	Standard error of mean		3.87	3.78	3.62	4.03
	95% Confidence interval for mean	Lower limit	99.46	90.08	88.32	90.06
		Upper limit	115.73	105.94	103.53	107.00
	Median		106.39	98.18	95.92	98.33
	Variance		284.98	270.84	248.89	308.96
	Std. deviation		16.88	16.46	15.78	17.58
	Minimum		80.21	68.90	72.08	72.42
	Maximum		144.04	131.80	120.58	126.22

Table 3 Frequency of bradycardia

	Heart rate		Chi-square: 8.437
Treatment arm	Bradycardia	Normal	p = 0.0037
ATG	97	2253	2350 (62.6%)
Steroid	32	1369	1401 (37.4%)
	129 3.40%	3622 96.60%	3751 (100%)

The frequency of bradycardic episodes was higher in the ATG than in the steroid alone group (p = 0.0039)

Discussion

Our retrospective study indicates that ATG therapy with steroid is associated with a definite risk of bradycardic episodes in children with SAA or in children receiving prophylaxis for GvHD. The retrospective nature and the great number of influencing factors prevent us to conclude a causative association between ATG and bradycardia. However, it is reasonable to speculate that the higher incidence of bradycardia in ATG group reflects a cardiac side effect.

It is now well established that antibodies may affect the function of the sinus node and the atrioventricular junction. While systemic lupus erythematosus (SLE) associated anti-SSA/Ro maternal autoantibody has been shown to be in association with sinus bradycardia in newborns [14, 15], both anti-SSA/Ro and anti-SSB/La antibodies of an SLE mother can cause atrioventricular heart block in the newborn [16–22]. There are also alloantibodies that may contribute to the rejection of the transplanted heart [23–25]. Based on these experiments a speculative explanation for our findings is that ATG preparations could contain antibodies with a cardiac action similar to anti-SSA, anti-SSB or any other type of alloantibodies and, therefore, may induce sinus bradycardia.

Other mechanisms contributing to bradycardia could be a systemic hemodynamic response to elevated intracranial pressure. However, we experienced no alteration in blood pressures before and after ATG administration, and none of our patients exhibited any sign or symptom of elevated intracranial pressure. No remarkable respiratory sinus arrhythmia or low blood pressure was observed, which should have directed our interest to exaggerated vagal activity. Since the sinus bradycardia developed in an isolated manner, this phenomenon is probably a specific cardiac adverse event.

Of note, we noticed no difference in bradycardia regarding the origin and type of the ATG product (However, the modest sample size (only 3 patients treated with ATGAM, 3 with ATG-Fresenius) prevented us to compare safety issues with these drugs).

ATG destroys human T-lymphocytes; therefore, a severe cytokine storm reaction occurs commonly during ATG administration. Our institutional protocol prescribes high-dose steroid premedication and this regime may also induce bradycardia. Indeed, a cohort analysis of 61 steroid treated children indicated that there is a risk of developing bradycardia during treatment with glucocorticoids for ALL or GvHD [26]. However, this effect of steroid therapy may be mediated by elevated intracranial pressure, and diastolic and arterial blood pressures also increased in these patients. Our results do not support these data, as no significant changes related to the above-mentioned steroid effect were observed during the therapy in any of the groups. Although we noticed the lowering of the heart rate after steroid alone treatment, this was not as profound as after combined ATG – steroid therapy. We hypothesize that since the steroid alone group has a systemic inflammatory disease (GvHD) associated with tachycardia (higher heart rates than in the ATG group pretreatment, as shown in Table 2 and Fig. 1), the initiation of the well-known effective glucocorticoid treatment for GvHD can decrease the heart rate to a normal range. On the contrary, ATG can decrease the normal heart rates and severe bradycardia can develop.

Some limitations of our study need to be considered. Patient population was heterogeneous in terms of diagnosis. It was a retrospective study with modest sample size. Data were collected from patients who had received ATG and steroid according to defined protocols. Since steroids are generally used with ATG administration in the transplant setting, further investigations would be difficult to perform.

Table 4 Frequency of severe bradycardia

	Heart rate		Fisher's exact
Treatment arm	Bradycardia	Normal	p = 0.0029
ATG	13	2337	2350 (62.6%)
Steroid	0	1401	1401 (37.4%)
	13 0.30%	3738 99.70%	3751 (100%)

Severe bradycardia was observed only in the ATG group (p = 0.0029)

Conclusion

Our retrospective study suggests that ATG administration might be associated with transient asymptomatic bradycardia, therefore close monitoring of the heart rate is recommended during and after ATG treatment. However, this seems to be a benign effect with no specific intervention required. Further studies are needed to determine the exact impact of ATG therapy on cardiac function.

Author Contributions All authors have contributed substantially in the conception and design of the study. They all participated actively in the writing and approving of the manuscript.

Compliance with Ethical Standards

Conflict of Interest The authors report no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study formal consent is not required.

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