Grafalon® vs. Thymoglobulin® as an Induction Agent in Renal Transplantation – A Retrospective Study

Abstract

Introduction: Antihuman thymocyte immunoglobulin, used as an induction agent in renal transplantation, is of two types – thymoglobulin and grafalon (formerly ATG-Fresenius). In this study, we compared outcomes with these two agents. Methods: This was a single-center retrospective study of patients transplanted from January 2017 to October 2019, who received either grafalon or thymoglobulin induction. Grafalon or thymoglobulin was given at 6 and 3 mg/kg, respectively, followed by standard triple immunosuppression of tacrolimus, MMF, and prednisolone. Results: Median follow up was 22 (3–36) months. Thymoglobulin was given to 255 patients, whereas 78 patients received grafalon. Baseline demographics were similar between the two groups although significantly more patients in the grafalon group received ABO incompatible transplant (15% vs. 4.3%; \( P = 0.002 \)). Patient survival was similar between the two groups (99% in grafalon vs. 98.8% in thymoglobulin; \( P = 1.0 \)). Death censored graft survival was also similar (99% in grafalon vs. 100% in thymoglobulin; \( P = 0.23 \)). Biopsy proven acute rejection (BPAR) was significantly higher in the grafalon group (12.8% vs. 5.1%, \( P = 0.04 \)). The significance persisted after multivariable regression analysis (\( P = 0.02 \)). Other outcomes such as infection rate and estimated glomerular filtration rate on last follow up were comparable between the two groups. Conclusions: Grafalon (6 mg/kg dose) when used as an induction agent was associated with significantly higher rate of BPARs as compared to thymoglobulin (3 mg/kg dose) although with comparable short-term patient and death censored graft survival, graft function, and infection rates.

Keywords: Antilymphocyte serum, India, kidney transplantation, rabbit, thymoglobulin

Introduction

Antithymocyte globulin (ATG) is a potent immunosuppressive agent used to prevent and treat rejections in renal transplantation.\(^1\) These are polyclonal lymphocyte depleting agents and work through three different mechanisms viz a) apoptosis via activation-induced cell death, b) antibody-dependent cell-mediated cytotoxicity, and c) complement-dependent cytotoxicity (CDC).\(^1\)\(^-\)\(^3\) There are two types of ATG preparations available. Both are prepared in rabbits. While grafalon\(^\circledR\) (Neovii Pharmaceuticals AG, Switzerland – formerly known as ATG-Fresenius or ATG-F) is produced by immunizing rabbits with the T-cell leukemia line Jurkat, thymoglobulin\(^\circledR\) (Sano-Aventis, Boston, USA) is produced by immunizing with human thymocytes. Both differ in the method of production and marketed formulations. Despite having common mechanism of actions, there are few differences as well. They have different antigen specificities. Grafalon has greater selectivity for activated T-cells and also depletes CD4+ CD28- T-cells.\(^4\)\(^,\)\(^5\)

Grafalon was introduced in India in 2016, and since then, it has been used in multiple centers across the country for induction and antirejection treatment. Thymoglobulin has been in use for this purpose since long time. Despite increasing use of grafalon, there are no studies comparing its efficacy and safety vis-à-vis thymoglobulin from India. Engineer \textit{et al.} published their initial experience with grafalon as an induction agent in kidney transplantation, but the number was small. Patients receiving grafalon had high biopsy proven acute rejection (BPAR) of 36.3%, but the dose of grafalon used (4 mg/kg) was lower.\(^6\) Even outside India there are very few studies comparing these two induction agents.\(^7\)\(^-\)\(^9\)

We hereby present our experience with

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grafalon and compare its outcomes with thymoglobulin as an induction agent in renal transplants.

**Methods**

This was a single-center retrospective study. The study was approved by the ethics committee (MICR-2003/2022). All the patients transplanted from January 2017 to October 2019 at our center who received either grafalon or thymoglobulin induction were included. Those patients who had any allergy to above drugs were excluded.

After complete donor and recipient evaluation immunological tests were done. Human leucocyte antigen (HLA) A, B, DRB1 typing was done for both. CDC and flow cytometry cross match were done for all. Patients with a positive CDC cross match were not transplanted, while those with a positive flow cytometry cross match underwent further evaluation with Luminex single-antigen bead assay to look for anti-HLA antibodies. HLA incompatibility was defined as the presence of a positive flow cytometry cross match (T cell mean channel shift >26; B cell mean channel shift >110) and presence of donor specific anti-HLA antibody (DSA) with mean fluorescence intensity (MFI) >1000.

**Immunosuppressive protocols**

Patients received the first dose of ATG on the day of transplant. Total dose of Grafalon given was 6 mg/kg IV (3 mg/kg each on postoperative day (POD) 0 and 2), while that of thymoglobulin was 3 mg/kg IV (1.5 mg/kg each on POD 0 and POD2). The first dose infusion was started intraoperatively before clamp release. All the patients also received 500 mg IV methylprednisolone intra-op followed by 100 mg of IV hydrocortisone 8th hourly on POD 0. Oral prednisolone was started at 40 mg daily from POD1 and tapered to 20 mg daily on discharge. Other maintenance immunosuppression (IS) consisted of tacrolimus and mycophenolate mofetil/sodium, which was started one day prior to transplant. Tacrolimus was started at a dose of 0.1 mg/kg in two divided doses, while mycophenolate mofetil (MMF/MMF-S) was started at 1 gm/720 mg twice daily. Pediatric patients received MMF at 600 mg/m².

Patients who underwent ABO incompatible renal transplant (ABOiRT) received IV rituximab 200 mg 2 weeks prior to the intended date of transplant. After a week of receiving rituximab, oral tacrolimus was started at 0.05 mg/kg/day in two equal divided doses and MMF-S at 720 mg twice daily. Cascade plasmapheresis or immunoadsorption was started at this point. Alternate day cascade plasmapheresis or immunoadsorption was continued till anti-blood group antibody titer of 1:8 was achieved. At this point, the transplant was done. Post-transplant course till discharge was similar as above. Desensitization in patients undergoing HLA incompatible transplant consisted of alternate day sessions of double filtration plasmapheresis to achieve DSA MFI <1000.

Patients were followed up weekly once for the first month, weekly twice for the second month, once in fortnight for the third month, and monthly once thereafter for a year. After 1-year, patients were followed up once in 2–3 months lifelong. During every visit, renal function tests and hemogram were monitored. Tacrolimus target trough level was 8–12 ng/ml during first 3 months, 5–8 ng/ml from 3 to 6 months, and <5 ng/ml thereafter. Prednisolone was tapered to 5 mg by the end of the third month. All patients received trimethoprim + sulfamethoxazole prophylaxis for 6 months, and valgancyclovir was given as cytomegalovirus (CMV) prophylaxis for 3 months. Estimated glomerular filtration rate (eGFR) was calculated by serum creatinine-based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula for adults, while for pediatric patients, it was calculated by bedside Schwartz equation. Statistical analysis was done using Prism GraphPad for Mac, version 8 (GraphPad Software, San Diego, California). Data were reported as mean values ± standard deviation. Continuous variables were compared using unpaired t-test, while categorical values were compared using Chi-square test or Fisher’s exact test. Survival analysis was performed by the Kaplan–Meier method and groups compared using the log rank-test. \( P < 0.05 \) was considered as statistically significant. Multivariable regression analysis was done to detect independent predictors of significant outcomes.

**Results**

During the study period, a total of 570 renal transplants were done. There were 255 patients who received thymoglobulin and 78 patients received grafalon as induction agent. Median follow up duration was 22 (3–36) months. All the transplants were living donor in both the groups, and no deceased donor transplant was done during the study period.

Baseline demographics are shown in Table 1. All the parameters were comparable between the two groups including the number of HLA incompatible transplants, but the grafalon group had higher number of ABOiRT. There was no significant difference in the mean DSA levels of HLA incompatible transplant recipients between the two groups (1202 ± 1699 in grafalon vs 3278 ± 3452 in thymoglobulin group; \( P = 0.44 \)). Seven out of the nine HLA incompatible transplant recipients in the thymoglobulin group, while one out of the two in the grafalon group received desensitization. This was not statistically significant (\( P = 0.49 \)).

Table 2 shows the patient outcomes. Patient survival and death censored graft survival were comparable between the two groups. One patient lost graft in the grafalon group due to drug noncompliance, while there was no graft loss in thymoglobulin group. BPAR rate was significantly higher in the grafalon group (12.8%) compared to the thymoglobulin group (5.1%), \( P = 0.04 \). There was one
case of antibody-mediated rejection (AMR) in the grafalon group, which was an HLA incompatible transplant. There was no case of AMR in the thymoglobulin group. The significant difference in BPAR between the two groups persisted ($P = 0.02$) after a multivariable Cox logistic regression analysis adjusting for recipient and donor age and gender, dialysis vintage, pre-emptive transplants, HLA mismatches, HLAi, and ABOiRT [Table 3].

There was no difference in the infection rate between the two groups and incidences of viral infections such as CMV and BK virus (BKV) were also comparable. Figures 1 and 2 shows the Kaplan Meier (KM) curves for patient and death censored graft survival, while Figure 3 is the KM curve for acute rejection free survival.

### Discussion

Induction agents reduce the acute rejections in renal transplant recipients. Lymphocyte depleting agent ATG is a potent-immunosuppressive agent. Studies comparing it with interleukin 2 receptor antagonist (IL2RA) have shown its superiority in preventing BPAR.\textsuperscript{[10-12]} Thymoglobulin has been the predominantly used ATG in most of the places although the use of grafalon has increased in India lately. Both share similar mechanism of action, but there are quite a few differences as well. They have different antigen specificities.\textsuperscript{[13,14]} There have been very few studies comparing these two agents as there are none from India. The present study compared outcomes with these two lymphocyte depleting induction agents.

### Table 1: Demographic characteristics and clinical profile of patients

| Variables                        | Grafalon ($n=78$) | Thymoglobulin ($n=255$) | $P$  |
|----------------------------------|-------------------|--------------------------|------|
| Recipient age (years)            | 43.1±12.1         | 41.1±12.5                | 0.21 |
| Donor age (years)                | 47.8±10.5         | 46.7±11.2                | 0.44 |
| Recipient gender                 | 72 M: 6 F (92%; 8%) | 214 M: 41 F (84%; 16%)  | 0.07 |
| Donor gender                     | 14 M: 64 F (21%; 79%) | 65 M: 190 F (25%; 75%)  | 0.22 |
| Dialysis vintage                 | 4.1±5.5           | 6.1±8.8                  | 0.06 |
| HLA mismatch                     | 4.1±1.2           | 4±1.4                    | 0.57 |
| ABO incompatible transplant      | 15% ($n=12$)      | 4.3% ($n=11$)            | 0.002* |
| HLA incompatible transplant      | 2.6% ($n=2$)      | 3.5% ($n=9$)             | 1    |
| Pre-emptive transplant           | 21.8% ($n=17$)    | 14.9% ($n=38$)           | 0.16 |

*Significant. HLA=Human leucocyte antigen

### Table 2: Patient outcomes

| Variables                      | Grafalon ($n=78$) | Thymoglobulin ($n=255$) | $P$  |
|--------------------------------|-------------------|--------------------------|------|
| Patient survival               | 99%               | 98.8%                    | 1    |
| Death censored graft survival  | 99%               | 100%                     | 0.23 |
| eGFR (on last follow up)(ml/min)| 73.1±20.5        | 75.9±23.8                | 0.35 |
| BPAR                           | 12.8% ($n=10$)    | 5.1% ($n=13$)            | 0.04*|
| Infections                     | 12.8% ($n=10$)    | 20.7% ($n=53$)           | 0.13 |
| CMV infection                  | 0                 | 1% ($n=2$)               | 1    |
| BKV infection                  | 2.5% ($n=2$)      | 0.4% ($n=1$)             | 0.14 |
| Post-transplant malignancy     | 0                 | 0                        | 1    |
| NODAT                          | 5.1% ($n=4$)      | 6.7% ($n=17$)            | 0.79 |

*Significant. BPAR=Biopsy proven acute rejection; CMV=Cytomegalovirus; eGFR=Estimated glomerular filtration rate; NODAT=New onset diabetes after transplant

### Table 3: Multivariable Cox regression analysis for variables associated with biopsy proven acute rejection

| Variable            | Estimate | Standard error | Odds ratio | 95% CI (profile likelihood) | $P$  |
|---------------------|----------|----------------|------------|-----------------------------|------|
| Grafalon            | 1.11     | 0.48           | 3.05       | 1.17-7.88                   | 0.02*|
| Recipient age       | -0.04    | 0.02           | 0.96       | 0.92-0.99                   | 0.04*|
| Male recipient      | -0.07    | 0.64           | 0.93       | 0.28-3.72                   | 0.91 |
| Donor age           | 0.03     | 0.02           | 1.03       | 0.99-1.08                   | 0.13 |
| Male donor          | 0.06     | 0.57           | 1.06       | 0.32-3.07                   | 0.92 |
| Dialysis vintage    | -0.03    | 0.04           | 0.98       | 0.89-1.04                   | 0.52 |
| HLA mismatch        | 0.28     | 0.21           | 1.32       | 0.88-2.01                   | 0.19 |
| HLA incompatible     | 1.98     | 0.77           | 7.21       | 1.38-31.15                  | 0.01*|
| ABO incompatible     | 0.71     | 0.73           | 2.03       | 0.41-7.77                   | 0.33 |
| Pre-emptive transplant | -0.59  | 0.72           | 0.56       | 0.11-2.02                   | 0.41 |

*Significant. HLA=Human leucocyte antigen
In the present study, dose of grafalon used was 6 mg/kg. The dose recommendation as per the monograph is 3–4 mg/kg/day for 5–14 days (totaling 10–70 mg/kg). The mean cumulative dose in a study of 422 living donor transplant patients (from 2009 to 2015) by Yilmaz et al. was 5.1 ± 2.7 mg/kg. In total, 18% of the patients had received <3 mg/kg, 78.2% had received dose of 3–12 mg/kg, and 3.3% had received >12 mg/kg.\[15\]

Patient survival in our study in the grafalon group was 99%, while in the thymoglobulin group was 98.8% \( (P = 1.0) \). The difference was not statistically significant. In a randomized controlled trial by Burkhalter et al. comparing thymoglobulin and grafalon in high immunological risk patients (total dose of grafalon used was 21 mg/kg and thymoglobulin was 6 mg/kg), the patient survival was comparable between the groups.\[7\] In another study by Ducloux et al., mortality was significantly higher in thymoglobulin group. He had analyzed the data on 194 consecutive transplant patients in France who got transplanted from 1993 to 2001. The dose of grafalon used was 21 mg/kg and thymoglobulin was 6 to 13 mg/kg.\[10\] Similarly, in a Bayesian network meta-analysis of randomized controlled trials done by Song et al., thymoglobulin was inferior when compared to ATG-F in preventing patient mortality.\[8\]

Death censored graft survival in our study was comparable between the two groups (99% in grafalon vs. 100% in thymoglobulin; \( P = 0.23 \)). In a retrospective study by Chen et al. in kidney transplant recipients from donation after cardiac death donors, the graft survival was comparable between the two groups.\[9\] In the study by Didier et al., the graft survival was comparable while the meta-analysis by Song et al. showed higher graft survival in the ATG-F group overall, but in high immunological risk, patients fared better with thymoglobulin.\[8,16\]

The BPAR was significantly higher in patients receiving grafalon in our study (12.8% vs. 5.1%; \( P = 0.04 \)). One patient had AMR in the grafalon group. He had undergone an HLA incompatible renal transplant. Various studies have shown the advantage of using thymoglobulin over ATG-F in preventing BPAR,\[8,9\] although in the study by Burkhalter et al. there was no difference between the two groups.\[7\] It is to be noted that in our study the grafalon group had higher number of ABOiRT. After a multivariate Cox logistic regression analysis adjusting for baseline variables, the difference in BPAR between the two groups was still significant \( (P = 0.02) \). There was no significant difference in the eGFR on last follow up between the two groups (73.1 ± 20.5 ml/min in grafalon vs. 75.9 ± 23.8 in thymoglobulin; \( P = 0.35 \)). In the study by Burkhalter et al., there was no difference in 2-year GFR between the two groups.\[7\]

Infection rate was comparable between the two groups in the present study (12.8% in grafalon vs. 20.7% in thymoglobulin; \( P = 0.13 \)). There was no difference in the incidence of CMV and BKV infections. Infection rates were comparable in the study by Burkhalter et al. although the BKV replication was significantly more frequent in the ATG-F group.\[7\] In the meta-analysis by Song et al., thymoglobulin was associated with higher risk of infections including CMV infections.\[8\] In the study by Ducloux et al., CMV disease was higher in the thymoglobulin group although not all the patient had received valgancyclovir prophylaxis.\[16\]

Various studies have shown higher incidence of post-transplant malignancy in patients receiving
thymoglobulin vs. those receiving ATG-F. In our study, none of the patients in either group developed post-transplant malignancy although the duration of follow-up was short. In the present study, incidence of new onset diabetes after transplant was comparable between the two groups. In the study by Song et al., it was higher in the thymoglobulin group.

We did a comparative cost analysis of the two medications. Grafalon's cost is Rs. 33000 per 100 mg (i.e., Rs. 330 per mg), while the cost of thymoglobulin is Rs. 13800 per 25 mg (Rs. 552 per mg). But as the dose of grafalon used was twice that of thymoglobulin, using grafalon was more expensive in the present study. So, grafalon at a dose of 6 mg/kg was not economical when compared to thymoglobulin at 3 mg/kg.

There are few limitations of the present study. It was a retrospective study and had heterogeneous patient population. The follow-up duration was short. No protocol biopsies or regular monitoring of CMV or BKV viremia was done. There was no routine monitoring of donor specific antibodies. Despite these limitations, this is the only study so far comparing grafalon with thymoglobulin from India. There is a need of randomized controlled trial with these two different ATG formulations in our patient population.

Concluding, when used for induction, grafalon (at a dose of 6 mg/kg) and thymoglobulin (at a dose of 3 mg/kg) have comparable outcomes in terms of patient and death censored graft survival, graft function as well as the rate of infections. Patients receiving grafalon had significantly higher rate of BPAR compared to thymoglobulin.

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Conflicts of interest
Dr. Vijay Kher has been in speaker forum for Zydus as well as Genzyme/Sanofi and has also received research grants from Genzyme. Dr. Ajay Kher has been in speaker forum for Sanofi. Other authors do not have any conflicts of interest associated with this publication.

References
1. Mohty M. Mechanisms of action of antithymocyte globulin: T-cell depletion and beyond. Leukemia 2007;21:1387-94.
2. Woodside KJ, Hu M, Gugliuzza KK, Hunter GC, Daller JA. T-lymphocyte apoptosis is increased by non-interleukin-2-dependent induction in human mixed lymphocyte cultures. Transplant Proc 2005;37:1949-52.
3. Genestier L, Fournel S, Flacher M, Assoussou O, Revillard JP, Bonnefoy-Berard N. Induction of Fas (Apo-1, CD95)-mediated apoptosis of activated lymphocytes by polyclonal antithymocyte globulins. Blood 1998;91:2360-8.
4. Shenton BK, White MD, Bell AE, Clark K, Rigg KM, Forsythe JL, et al. The paradox of ATG monitoring in renal transplantation. Transplant Proc 1994;26:3177-80.
5. Dufner C, Dejaco C, Hengster P, Bijuklic K, Joannidis M, Margreiter R, et al. Apoptotic effects of antilymphocyte globulins on human pro-inflammatory CD4+CD28- T-cells. PLoS One 2012;7:e33939.
6. Engineer DP, Patel H, Kute V, Shah P. Initial experience with Grafalon as induction agent in kidney transplantation. J Clin Diagn Res 2018;12:19-23.
7. Burkhalter F, Schaub S, Bucher C, Gurke L, Bachmann A, Hopfer H, et al. A comparison of two types of rabbit antithymocyte globulin induction therapy in immunological high-risk kidney recipients: A prospective randomized control study. PLoS One 2016;11:e0165233.
8. Song T, Yin S, Li X, Jiang Y, Lin T. Thymoglobulin vs. ATG-Fresenius as induction therapy in kidney transplantation: A bayesian network meta-analysis of randomized controlled trials. Front Immunol 2020;11:457.
9. Chen G-D, Lai X-Q, Ko DS-C, Qiu J, Wang C-X, Han M, et al. Comparison of efficacy and safety between rabbit anti-thymocyte globulin and anti-T lymphocyte globulin in kidney transplantation from donation after cardiac death: A retrospective cohort study. Nephrology 2015;20:539-43.
10. Brennan DC, Daller JA, Lake KD, Cibrik D, Del Castillo D. Thymoglobulin induction study. G. Rabbit antithymocyte globulin versus basiliximab in renal transplantation. N Engl J Med 2006;355:1967-77.
11. Noel C, Abramowicz D, Durand D, Mourad G, Lang P, Kessler M, et al. Daclizumab versus antithymocyte globulin in high-immunological-risk renal transplant recipients. J Am Soc Nephrol 2009;20:1385-92.
12. Alloway RR, Woodie ES, Abramowicz D, Segev DL, Castan R, Isley JN, et al. Rabbit anti-thymocyte globulin for the prevention of acute rejection in kidney transplantation. Am J Transplant 2019;19:2252-61.
13. Popow I, Leitner J, Grabmeier-Pftershammer K, Majdic O, Zlabinger GJ, Kundi M, et al. A comprehensive and quantitative analysis of the major specificities in rabbit antithymocyte globulin preparations. Am J Transplant 2013;13:3103-13.
14. Bourdage JS, Hamlin DM. Comparative polyclonal antithymocyte globulin and antilymphocyte/antilymphoblast globulin anti-CD antigen analysis by flow cytometry. Transplantation 1995;59:1194-200.
15. Yilmaz M, Sezer TO, Gunay E, Solak I, Celtik A, Hoscoskun C, et al. Efficacy and safety of ATG-Fresenius as an induction agent in living-donor kidney transplantation. Transplant Proc 2017;49:481-5.
16. Ducloix D, Kazory A, Challier B, Coutet J, Bresson-Vaurrin C, Motte G, et al. Long-term toxicity of antithymocyte globulin induction may vary with choice of agent: A single-center retrospective study. Transplantation 2004;77:1029-33.