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A Proposal for Early Dosing Regimens in Heart Transplant Patients Receiving Thymoglobulin and Calcineurin Inhibition

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There is currently no consensus regarding the dose or duration of rabbit antithymocyte globulin (rATG) induction in different types of heart transplant patients, or the timing and intensity of initial calcineurin inhibitor (CNI) therapy in rATG-treated individuals. Based on limited data and personal experience, the authors propose an approach to rATG dosing and initial CNI administration. Usually rATG is initiated immediately after exclusion of primary graft failure, although intraoperative initiation may be appropriate in specific cases. A total rATG dose of 4.5 to 7.5 mg/kg is advisable, tailored within that range according to immunologic risk and adjusted according to immune monitoring. Lower doses (eg, 3.0 mg/kg) of rATG can be used in patients at low immunological risk, or 1.5 to 2.5 mg/kg for patients with infection on mechanical circulatory support. The timing of CNI introduction is dictated by renal recovery, varying between day 3 and day 0 after heart transplantation, and the initial target exposure is influenced by immunological risk and presence of infection. Rabbit antithymocyte globulin and CNI dosing should not overlap except in high-risk cases. There is a clear need for more studies to define the optimal dosing regimens for rATG and early CNI exposure according to risk profile in heart transplantation.

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Rabbit antithymocyte globulin (rATG) is widely used for induction therapy in adult and pediatric heart transplant recipients. Despite the fact that rATG has been

available for more than 30 years, new roles are being found for its use, for example, in supporting early minimization of calcineurin inhibitor (CNI) exposure or to reduce the risk of

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rejection in presensitized transplant candidates.¹ Two rATG products are commercially available: Thymoglobulin and ATG-Neovii (formerly ATG-Fresenius). Thymoglobulin is a rabbit antihuman thymocyte immunoglobulin. ATG-Neovii is an anti-T-lymphocyte immunoglobulin derived from rabbits immunized with Jurkat cells, a lymphoblastoid cell line. The 2 products cannot be considered interchangeable, with differing dosing regimens and evidence that they exhibit different hematologic profiles.^{2,3} Thymoglobulin is more widely used and documented than ATG-Neovii, and “rATG” will here refer to Thymoglobulin unless otherwise stated.

In the early 2000s, rATG (Thymoglobulin) induction regimens in heart transplant patients delivered a total dose of up to 10.5 to 15 mg/kg in clinical trials^{4,6} but high rates of hematological side effects and infectious complications prompted substantial dose reductions since that time. By the late 2000s, a standard rATG protocol did not usually exceed 7.5 mg/kg,⁷⁻⁹ compatible with modern dosing in kidney transplantation.¹⁰ Currently, rATG is generally dosed according to body weight, with caution applied if the patients weigh less than 40 kg or more than 80 kg. Adjusting the dose according to the pharmacodynamic response, based on CD3+ T-cell count may lower the total dose (and drug costs) while maintaining lymphocyte suppression,^{11,12} but randomized trials are lacking, and data in heart transplants remain limited.^{13,14} Moreover, immunological results must be provided within 12 hours to allow adjustment of the following dose, which is often impractical.

There is currently no consensus, however, regarding the optimal dose or duration of rATG induction in heart transplant patients, or how it should be amended according to patient's risk profile or the type of maintenance immunosuppression regimen. The manufacturers' guidance on dosing for rATG provides little guidance. For Thymoglobulin, the licence states a recommended total dose in heart transplantation ranges from 3 mg/kg to 12.5 mg/kg, given over 3 to 5 days. For ATG-Neovii, an even wider range is included in the licensed recommendations: 2 to 5 mg/kg/day for between 5 and 14 days.

We previously proposed an algorithm for the use of rATG in heart transplant patients in a variety of circumstances, including suggested strategies for CNi exposure in each setting.¹⁵ In the current article, we have sought to develop proposals for dosing protocols for rATG, and for CNi agents during the first postoperative month, in these various situations. Where possible, these are based on published studies, but few well-designed trials of rATG with CNi in heart transplantation are available. Where necessary, proposals are therefore also derived from the authors' many years of clinical experience with rATG induction. In addition, 15 heart transplant centers in Germany, Austria, and Switzerland were sent a questionnaire by the authors requesting information about current prescribing practices for rATG in heart transplant recipients.

Impact of rATG Dose on Lymphocyte Count

Rabbit antithymocyte globulin contains a wide range of T cell and other antibody specificities.¹⁶ It acts primarily via T-cell depletion, with preferential recovery of CD4⁺CD25^{high}Foxp3⁺ regulatory T cells,¹⁷⁻¹⁹ but also contains antibodies against antigens on natural killer cells, B cells and plasma cells, and against adhesion molecules and chemokine receptors.²⁰ Animal data indicate that T-cell apoptosis in peripheral blood occurs even at low concentrations

of rATG,²¹⁻²³ but depletion of B cells and natural killer cells may become relevant only at high doses.²¹

Evidence from animal models²¹ and clinical kidney transplantation^{24,25} has demonstrated the dose dependency of rATG-induced T-cell depletion. A prospective study of 40 kidney transplants found that a very low total dose of rATG (1.5 mg/kg) permitted recovery of the T-cell count by month 3 posttransplant, whereas a total dose of 6.0 mg/kg suppressed the count for up to 1 year compared with controls.²⁴ For kidney transplant patients, it appears that a total dose of 6 mg/kg effectively depletes lymphocytes in peripheral blood²⁶ and may be the minimum required to prevent rejection,^{27,28} although lower doses have proved effective in low-risk populations.^{24,29} In heart transplantation, a retrospective single-center study compared lymphocyte depletion and recovery in 105 patients given seven 1.5-mg/kg doses of rATG (total, 10.5 mg/kg) versus 39 patients given 5 doses (total, 7.5 mg/kg). Lymphocyte count decreased rapidly in both groups but was significantly higher in the lower-dose cohort during days 7 to 21, after which counts were similar to month 12⁹ (Figure 1). The proportion of patients reaching the target absolute lymphocyte count of 200 per μ L was achieved by 62% and 37% of the higher-dose and lower-dose groups, respectively, by day 7 ($P = 0.009$). Studies in stem cell transplantation have confirmed that lower total exposure to rATG is associated with faster recovery of T-cell counts.³⁰

The split of the rATG dose, or whether dosing is started intraoperatively or perioperatively, does not seem to be particularly influential. Limited data with rATG in kidney transplantation²⁶ and with ATG-Neovii in heart transplantation³¹ have not indicated any substantive difference in the depletion or recovery of different T-cell subpopulations when the majority of the total dose is given earlier (eg, in a large initial infusion), or if a shorter dosing period is used. In kidney transplantation, 1 randomized trial found a lower risk of delayed graft function using intraoperative rATG versus postoperative rATG,³² and there is some evidence for reduced ischemia-reperfusion injury with intraoperative rATG in liver transplant patients.³³ However, there are no published data addressing the question of whether preoperative or intraoperative dosing of rATG lowers the risk for primary graft failure after heart transplantation.

Impact of rATG Dose on Safety

The key safety concerns for lymphocyte-depleting induction are infections and malignancy. Early high-dose rATG

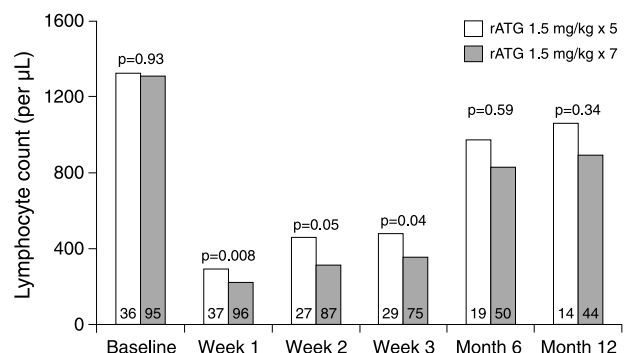


FIGURE 1. Change in mean absolute lymphocyte count in a retrospective, single-center study of heart transplant patients receiving rATG 1.5 mg/kg for 7 days ($n = 107$) or 5 days ($n = 39$).⁹

regimens (total dose, 12.5 mg/kg) in kidney transplantation increased the risk for infections, particularly for cytomegalovirus (CMV) infection at a time when CMV prophylaxis was not widely used.^{34,35} As would be expected, this is a dose-dependent effect. In liver transplantation, a very high total rATG dose of 25 mg/kg over 10 days resulted in a significantly higher rate of fatal infections than in patients given a more normal dose of 7.5 mg/kg (34.6% vs 15.5%, $P = 0.01$).³⁶ In a randomized trial of 80 heart transplant recipients, Mattei et al³⁷ showed a higher rate of infectious death with rATG induction versus basiliximab when a total rATG dose of up to 12.5 mg/kg was administered. A retrospective study in 40 heart transplant patients, also treated with rATG doses of up to 12.5 mg/kg, reported a higher rate of bacterial infections as compared with induction with the interleukin-2 receptor antagonist daclizumab.³⁸ Such high doses of rATG would not now be considered advisable. Separately, it should also be noted that a warning letter was issued in 2014 highlighting that basiliximab is not licensed for use in heart transplantation.³⁹ Daclizumab is no longer commercially available.

A retrospective single-center analysis of 523 heart transplant patients by Aliabadi et al⁴⁰ analyzed infection rates in the subgroup of patients given a total rATG dose of less than 4.5 mg/kg, 4.5 to 7.5 mg/kg, or greater than 7.5 mg/kg. Kaplan-Meier estimates indicated the 10-year incidence of severe infection to be 37%, 23%, and 45%, respectively ($P < 0.001$). On multivariate analysis, the cohort given 4.5 to 7.5 mg/kg had a reduced risk of severe infection compared with the highest-dose group (hazard ratio [HR], 1.71; $P = 0.015$) as might be expected, but also compared with the lowest-dose group (HR, 1.86; $P = 0.011$).⁴⁰ This may have been due to the fact that higher rates of rejection under low-dose rATG necessitated intensive antirejection therapy, or that in patients who developed infection rATG therapy was discontinued. Cytomegalovirus infection was estimated to occur in 20%, 23%, and 35% by year 2 ($P = 0.009$), with CMV disease in 5%, 6%, and 23% ($P = 0.015$). Based on these data, an rATG dose in the range of 4.5 to 7.5 mg/kg would seem to be advisable with respect to infection risk.

Regarding malignancy, the rarity of events makes an accurate assessment of the impact of specific rATG doses difficult. In the retrospective study by Aliabadi et al,⁴⁰ in which patients were followed up for a median of 104 months, Kaplan-Meier estimates for freedom from malignancy did not differ between the 3 rATG dose groups (<4.5 mg/kg, 4.5-7.5 mg/kg, or >7.5 mg/kg) but the time to tumor development was significantly shorter in the high-dose cohort. A recent systematic review concluded that the risk of posttransplant lymphoproliferative disease (PTLD) is not influenced by rATG dose across all organ types or specifically in heart transplantation.⁴¹ No difference in risk was observed between a total dose of less than 5 mg/kg or 5 to 7 mg/kg, or between less than 7.5 mg/kg and 7.5 mg/kg or greater.⁴¹ Use of antiviral prophylaxis, however, showed a clear inverse association with risk for PTLD, and it may be that an effect of more intensive rATG is now less relevant in the era of widespread antiviral administration. In children, who are at particular risk for PTLD, there is limited evidence that additional rATG doses are a risk factor for PTLD⁴² and a cautious approach with a maximum dose of 3 to 4.5 mg/kg may be advisable.

rATG Dosing in Adults: Evidence From the Literature

Studies of rATG with immediate CNI (ie, from day 0 or day 1 posttransplant) have tended to use higher doses of rATG than currently, with higher rates of infection than with interleukin-2 receptor antagonist induction^{37,38} (Table 1). One interesting exception is a randomized trial by Yamani et al,⁸ in which low-risk heart transplant patients received rATG at a total dose of 6 mg/kg with immediate tacrolimus and mycophenolate mofetil but no oral steroids, a regimen that achieved a low rate of rejections (renal function was similar to a standard steroid-containing regimen). Confirmatory data are lacking.

Giving rATG induction with delayed CNI is now more usual, as clinicians seek to preserve renal function. Goland et al⁹ administered rATG at a dose of 1.5 mg/kg for 5 days (total dose, 7.5 mg/kg) in patients at standard risk for rejection, with CNI started from day 5. The incidence of biopsy-proven acute rejection (BPARG) was 20% at 1 year. This was significantly more frequent than that in patients known to be at high immunological risk, who received a relatively high total dose of 10.5 mg/kg, but there were no infection-related deaths in the lower-dose cohort (0/39) compared with 4 of 105 in the high-dose group. Renal data beyond week 1 were not reported. Another retrospective analysis, using a total rATG dose of only 4.5 mg/kg per day with cyclosporine (CsA) started at a mean of 5.3 days posttransplant, reported an acceptable rate of BPARG grade 3A or higher (6 episodes by month 6 in 23 patients) in an unselected cohort of heart transplant patients.⁷ The rate of rejection was similar to that in a previous group of 25 patients given basiliximab induction with CsA started early (mean, 2.2 days), but neither was creatinine clearance different between the 2 groups during the 1-year follow-up.⁷ In kidney transplantation, studies of patients receiving CNI and steroid maintenance therapy now tend to use a dose of approximately 6 mg/kg, with higher doses (up to 8.75 mg/kg) in sensitized patients,⁴³ but there are reports of successful outcomes using lower doses (eg, 3.75 mg/kg) in low-risk^{44,45} or unselected⁴⁶ patients.

The retrospective analysis by Aliabadi et al⁴⁰ is helpful regarding the association between rATG dose and immunosuppressive efficacy in patients given delayed CNI. The mean time to start of CsA or tacrolimus was 4.3 days and 4.9 days, respectively. Results showed a trend to more frequent acute rejection in unselected patients given a total dose less than 4.5 mg/kg compared with 4.5 to 7.5 mg/kg (HR, 1.98; $P = 0.057$), with deaths due to rejection in 14% and 3% of patients, respectively. There was a trend on multivariate analysis to lower survival in the group given less than 4.5 mg/kg versus 4.5 to 7.5 mg/kg (HR, 1.56; $P = 0.081$), whereas the medium-dose and high-dose groups showed similar survival rates (HR, 0.99 for 4.5-7.5 mg/kg vs >7.5 mg; $P = 0.984$). Using a combined endpoint of death, treated rejection, or severe infection, the group receiving a dose of 4.5 to 7.5 mg/kg showed the most favorable outcome ($P = 0.017$). These data suggest that a total rATG dose below 4.5 mg/kg in an unselected population is inadvisable if CNI initiation is delayed.

Time to Start of CNI Therapy: Evidence From the Literature

Studies describing outcomes in rATG-treated heart transplant patients have used various criteria for CNI initiation. The starting date for CNI therapy has been predefined for a

TABLE 1.**Studies of rATG induction and standard CNi therapy in adult heart transplant recipients**

| Study | Design/follow-up | Population | n | Induction dose and duration | Total rATG dose | CNI regimen | Adjunctive therapy | Acute rejection | Comment |
|-----------------------------------|---|---|----|---|----------------------|--|--------------------|---|---|
| Faggian et al, 2010 ³¹ | Randomized prospective single center, 5 y | Excluded patients with thrombocytopenia or leukopenia or active infection | 14 | ATG-Neovii, 9 mg/kg intraoperatively, 3 mg/kg × 3 | ATG-Neovii, 18 mg/kg | Immediate CsA | AZA, steroids | 65% vs 55% acute rejection by year 5 (n.s.) | No difference in infections |
| Mattei et al, 2007 ³⁷ | Randomized prospective multicenter, 6 mo | Low/moderate risk, with stable hemodynamic status and SCr ≤250 μmol/L | 42 | ATG-Neovii, 3 mg/kg × 7 | ATG-Neovii, 21 mg/kg | Immediate CsA | MMF, steroids | 45.2% vs 50% BPAR grade ≥1B (n.s.) 7/1% vs 18.4% BPAR grade ≥3A (P = 0.068) | Higher rate of infectious death with rATG (14.3% vs 0%, 0.027) |
| Carlson et al, 2005 ³⁸ | Retrospective single center, 1 y | Unselected consecutive cohort (with ≥4 wk survival posttransplant) | 20 | rATG 2.5 mg/kg × 3-5 d | 7.5-12.5 mg/kg | Immediate CsA (reduced exposure to month 3 in rATG cohort) | AZA, steroids | 20 vs 32 episodes BPAR grade ≥1 by month 3 (P = 0.07) due to more grade 1 episodes (P = 0.04) | Increased bacterial infections with rATG (85% vs 10%, P = 0.05) |
| Steroid avoidance | | | | | | | | | |
| Yamani et al, 2008 ⁸ | Randomized prospective single center, 1 y | Low risk for rejection ^c | 16 | rATG 1.5 mg/kg × 1 ^d , 0.9 mg/kg × 5 | 6 | Immediate TAC | MMF, no steroids | Mean, 0.81 vs 1.07 episodes | |
| | | | 16 | rATG 1.5 mg/kg ^d × 1 | 1.5 | | MMF, Steroids | ≥3A (n.s.) | |

^a Not commercially available.^b Adjusted according to predefined hematological criteria.^c Non-HLA-sensitized, panel-reactive antibodies <10%, negative T-cell and B-cell cytotoxicity, negative donor-specific HLA crossmatch on flow cytometry.^d First dose given intraoperatively.

AZA, azathioprine; MMF, mycophenolate mofetil; n.s., nonsignificant; SCr, serum creatinine; TAC, tacrolimus.

TABLE 2.
Studies of rATG induction and delayed or low/no CNI therapy in adult heart transplant recipients

| Study | Design/follow-up | Population | n | Induction dose and duration | Total rATG dose | CNI regimen | Adjunctive therapy | Outcome | Comment |
|---|---|---|-----|--|-----------------|--|----------------------|--|---|
| Delayed CNI Alibadi et al, 2015 ⁴⁰ | Retrospective single center; mean, 104 mo | Minimum survival 7 d; no high-risk retransplants | 100 | rATG <4.5 mg/kg | <4.5 mg/kg | CsA (started at mean 4.3 d) or TAC | Steroids | HR for any BPAR or treated rejection for 4.5 mg/kg vs 4.5-7.5 mg/kg: 1.98 (P = 0.057) | 1-y survival: 80%, 90%, 88%, respectively (HR 1.56, P = 0.081 for <4.5 mg/kg vs 4.5-7.5 mg/kg) |
| | | | 165 | rATG 4.5-7.5 mg/kg | 4.5-7.5 mg/kg | (started at mean 4.9 d) | | | |
| | | | 258 | >7.5 mg/kg | >7.5 mg/kg | | | | |
| Goland et al, 2008 ⁹ | Retrospective single center, 3 y | High-risk for rejection ^a or impaired renal function Normal risk for rejection, normal renal function | 105 | rATG 1.5 mg/kg × 7 d | 10.5 mg/kg | CsA or TAC started from day 7 | MMF or AZA, steroids | 7% vs 20% BPAR grade ≥1B at 1 year (P = 0.007) | Higher rATG dose prevented BPAR in high-risk population without increased CMV or viral infections |
| | | | 39 | rATG 1.5 mg/kg × 5 d | 7.5 mg/kg | CsA or TAC started from day 5 | | | |
| Flaman et al, 2006 ^{7b} | Retrospective single center, 1 y | Unselected, consecutive cohort | 23 | rATG 1.5 mg/kg × 3 | 4.5 mg/kg | CsA started mean 5.3 d | MMF, steroids | 6 vs 17 BPAR episodes ≥3A by month 6 (P = 0.023) | No difference in infections |
| | | | 25 | Basiliximab 20 mg × 2 | — | CsA started mean 2.2 d | | | |
| Schneitzler et al, 2002 ⁴ | Randomized prospective single center, 1 y | Consecutive first transplants; excluded patients with severe thrombocytopenia or infection | 24 | rATG 2.5 mg/kg × 5 ^a | 12.5 mg/kg | CsA days 1-3, depending on renal and liver function | AZA, steroids | Mean, 2.46 vs 2.63 rejections per patient (n.s.), with similar time to first rejection | No difference in infections |
| | | | 24 | ATG-Neovii, 3.0 mg/kg × 5 ^a | 15 mg/kg | CsA started at mean 7.3 d | | | |
| Delgado et al, 2005 ⁴⁹ | Retrospective single center, 1 y | Consecutive cohort (with ≥2 d survival posttransplant) with renal dysfunction (SCR ≥150 μmol) | 15 | rATG 1.5 mg/kg every 2 to 5 d until SCR <150 μmol ^b | Mean, 6.1 mg/kg | CsA started when SCR <150 μmol; mean, 12 d | MMF, steroids | 27% vs 59% BPAR episodes ≥3A (n.s.) | Similar SCR between groups after month 1 |
| | | | 17 | rATG 1.5 mg/kg for 5-7 d ^b | Mean, 7.0 mg/kg | CsA started at mean 2 d | | | |
| De Santo et al, 2004 ⁵ | Randomized prospective single center, 3 y | Standard immunological risk | 20 | rATG 2.5 mg/kg × 5 d | 12.5 mg/kg | CsA started after hemodynamic stabilization and normal renal function ^c | AZA, steroids | 25% vs 20% BPAR (P = 0.50) | No difference in rates of early (<6 mo) BPAR, severity or time to first rejection |
| | | | 20 | ATG-Neovii 2.5 mg/kg × 7 d | 17.5 mg/kg | | | | |

Continued next page

TABLE 2. (Continued)

| Study | Design/follow-up | Population | n | Induction dose and duration | Total rATG dose | CNI regimen | Adjunctive therapy | Outcome | Comment |
|--------------------------------------|--|--------------------------------|----|-------------------------------------|-----------------|---|---|--------------------------------|---|
| Low-exposure CNI/CNI-free | | | | | | | | | |
| Andreassen et al, 2014 ⁵⁰ | Randomized prospective multicenter, 1 y | Low-to-moderate rejection risk | 56 | rATG 1-2 mg/kg per day × 3-5 d | 3-10 mg/kg | Low CsA started by day 5, withdrawn at weeks 7-11 | Everolimus, MMF, steroids | BPAR ≥2A 40% vs 18% (P < 0.05) | Significantly higher measured GFR with CsA minimization |
| Meiser et al, 2005 ⁵¹ | Retrospective single center; mean, 241 d | Unselected | 59 | ATG-Neovii 6 mg/kg × 1, 3 mg/kg × 3 | 15 mg/kg | CsA started by day 5 | MMF, steroids Sirolimus, MMF, steroids | 25% BPAR | Stable SCr from week 2 onward |

^a African American, age <60 years, multiparous women, panel reactive antibody >10%, positive crossmatch, multigraft transplant.

^b Adjusted according to predefined hematological criteria.

^c Day of CsA introduction not stated.

GFR, glomerular filtration rate.

specific day posttransplant in several reports, but without consistency: starting dates have included day 2,⁴⁷ day 2 only if hemodynamically stable,⁶ day 3,⁴⁸ before day 5,^{9,49,50} on day 5,⁷ or day 7.⁹ Clinical triggers have also been used to determine the point of CNI introduction (eg, decline of pretransplant serum creatinine ≥ 150 μmol to <150 μmol posttransplant⁶ or after hemodynamic stabilization and normalization of renal function⁵). Usually, CNI therapy is started within 2 to 7 days posttransplant (Table 2). In the recent multicenter study, Scandinavian Heart Transplant Everolimus de novo Study With Early Calcineurin Inhibitors Avoidance, in which all patients received rATG, the study protocol stipulated that CsA could be started according to local practice but no later than day 5. One study from 2004 only started CNI at a mean of 12 days posttransplant, triggered by serum creatinine declining to less than 150 μmol in patients with pretransplant renal impairment and achieved a low rate of BPAR 3A or higher with a mean rATG dose of 6.1 mg/kg.⁶ Supporting data with such a long delay have not been published.

Proposed rATG Dosing Strategy in Adults

Current Practice

Fifteen heart transplant centers provided information regarding their use of rATG. Two centers do not use rATG. An overview of current practice at the remaining 13 centers in terms of dosing regimens for rATG and starting times for CNI therapy is summarized in Table 3.

Timing of rATG Introduction

In the majority of cases, rATG is initiated immediately after the transplant procedure. The first dose should be delayed for 1 to 2 hours after return to the intensive care unit to confirm that there is no bleeding and that the patient is hemodynamically stable. In cases of primary graft failure with extracorporeal membrane oxygenation, pulmonary

TABLE 3.

Overview of dosing regimens for rATG induction and CNI therapy in rATG-treated heart transplant patients based on questionnaire results

| Parameter | Category | No. centers |
|--|------------------------------|----------------|
| Timing of rATG initiation | Preoperatively | 1 |
| | Intraoperatively | 2 |
| | Postoperatively ^a | 10 |
| | Not stated | 1 |
| Duration of rATG therapy, d | 1 | 4 |
| | 2-5 | 9 |
| | 7-10 | 2 |
| Total dose of rATG, mg/kg | <4.5 | 6 |
| | 4.5-7.5 | 7 |
| | >7.5 | — |
| Timing of CNI initiation posttransplant, d | 1-3 | 8 ^b |
| | 3-5 | 3 |
| | 7-10 | 3 |

Data are shown from 14 heart transplant centers which currently use rATG in Germany (11), Switzerland (2) and Austria (1). Two additional centers in Germany who were sent a questionnaire do not use rATG

^a Varying between 2 and 8 to 16 hours after arrival in intensive care unit (or 6 hours after aortic clamping).

^b Including day 1 if no ATG (n = 2) or if GFR >50 mL/min (n = 1); initiation dependent on renal function (n = 3) or fixed initiation of CsA (day 1, n = 1) or tacrolimus on day 2 (n = 2) or day 3 (n = 1).

hypertension should be excluded as a cause of right heart failure. If the patient does not respond to treatment for pulmonary hypertension within 24 hours, rATG can be started at a dose of 1.5 mg/kg per day (2.5 mg/kg per day if thrombocyte count is high [at least >100 000 and preferably >150 000/mm³], subsequently adjusted by thrombocyte count).

It may potentially be advantageous to start rATG intraoperatively in patients at high risk of primary graft failure, for example, recipients of a heart from an expanded criteria donor, presensitized patients or those with a long ischemia time (>3-4 hours), although this has not yet been assessed clinically. Beiras et al⁵² have recently confirmed that rATG is not filtered out of the bloodstream during mechanical support. If rATG is started intraoperatively, a suitable protocol would be to initiate a 12-hour infusion either before transplant or after initiation of anesthesia (with mechanical ventilation and hemodynamic monitoring established) and introduction

of intravenous cardiovascular lines. Thrombocyte count and function must be monitored closely, for example, every hour during surgery and 4 times per day afterward, requiring involvement of the anesthesiologist.

Defining the rATG Dose

A total dose in the range 4.5 to 7.5 mg/kg is considered advisable in most cases. This is likely to be given over 3 to 5 days, but can be prolonged for up to 10 days if the dose is lowered or if administration is interrupted. An initial dose of 1.5 mg/kg is frequently used. Dosing must be adjusted as necessary based on hematology (see *Indications to amend or stop rATG administration* below). Accordingly, for twice-daily administration blood results must be available within 12 hours.

For patients at low immunological risk, a slightly lower total dose can be used (eg, 3.0-4.5 mg/kg) (Figure 2A). For standard-risk patients, the dose is likely to be in the range

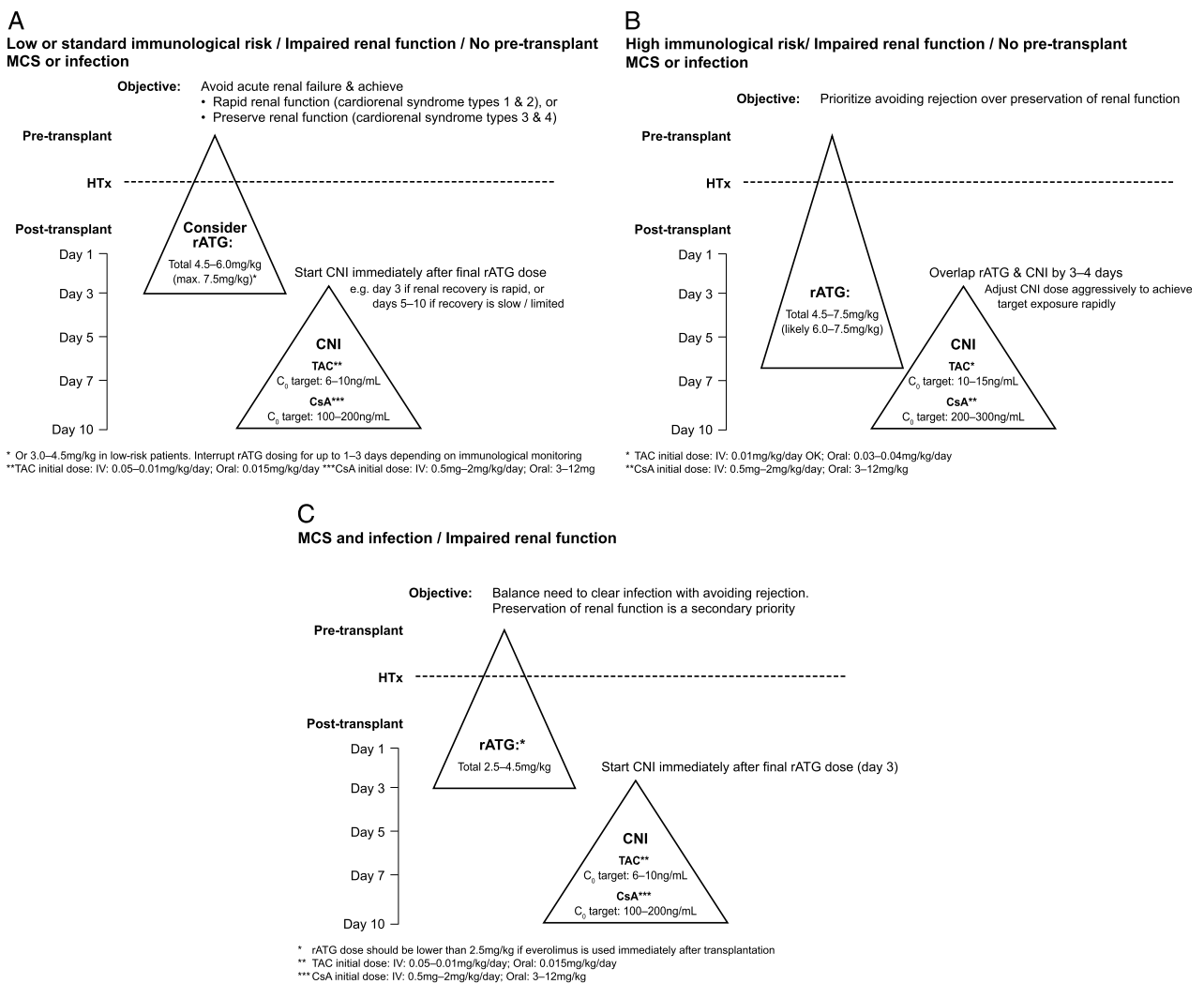


FIGURE 2. Suggested early dosing strategies for adult heart transplant patients receiving rATG and CNI therapy. A, Low or standard immunological risk patients with impaired renal function, with no pretransplant MCS or infection. B, High immunological risk patients with impaired renal function with no pretransplant MCS or infection. High immunological risk includes pretransplant DSA, black race; postpartum females; younger age (eg, <35 years). C, Patients on MCS with infection and impaired renal function. Patients should remain on the anti-infective regimen assigned pretransplant for 10 to 14 days posttransplant. Monitor closely for clinical and histological signs of rejection and increase exposure if required. If infection clears, immunosuppression can be increased. Renal function can be regarded as impaired if estimated GFR is below 50 to 60 mL/min per 1.73 m². Cardiorenal syndrome: type 1, abrupt worsening of cardiac function leading to acute kidney injury; type 2, chronic abnormalities in cardiac function causing progressive chronic kidney disease; type 3 abrupt worsening of renal function causing acute cardiac dysfunction; type 4, chronic kidney disease contributing to decreased cardiac function, cardiac hypertrophy, and/or increased risk of adverse cardiovascular events. DSA, donor-specific antibody.

4.5 to 6.0 mg/kg and should not exceed 7.5 mg/kg. In some cases, centers may apply only a single 1.5 mg/kg infusion of rATG if the clinical situation dictates, for example, if the lymphocyte count is low ($<200/\text{mm}^3$) or if adverse events develop, but the limited literature indicates that this is inadequate to control rejection adequately.⁸

In patients at high immunological risk (eg, pretransplant donor-specific antibodies, black race, postpartum females, younger age such as <35 years), the total rATG dose should certainly not be less than 4.5 mg/kg (although possibly 3.5 mg/kg in children), and 6.0 to 7.5 mg/kg is likely to be appropriate (Figure 2C).

In patients on pretransplant mechanical circulatory support (MCS) with infection, a dose between 1.5 and 2.5 mg/kg can be considered, but rATG may not be appropriate in this setting if the patient is extremely frail.

Caution is advised with the use of rATG in patients receiving everolimus, CNI, and steroids, based on the observation from the A2310 study that this regimen was associated with a higher rate of early (<3 months) infectious deaths, particularly in patients on pretransplant MCS,⁵³ indicating overimmunosuppression. If rATG is used in patients given everolimus from the time of transplant, a low rATG dose should be given (eg, total dose 1.5 to 2.5 mg/kg).

Duration of rATG Infusion

Anecdotal evidence suggests that a short infusion period for rATG (eg, 4-6 hours) exaggerates the risk of side effects, such as fever and thrombocytopenia. The first dose should be infused over no less than 6 hours, and infusion of rATG over 8 to 12 hours is advisable especially during the early postoperative period.

Indications to Amend or Stop rATG Administration

Rabbit antithymocyte globulin dosing should be stopped in the event of an allergic reaction (fever, hypotension) or if there are clinical and laboratory signs of infection or sepsis. Frequent hematological monitoring is mandatory. Heart transplant patients are vulnerable to postoperative thrombocytopenia due to the thrombocytopenic effects of circulatory bypass. Therefore, particular attention must be paid to thrombocyte count. Rabbit antithymocyte globulin dose should be lowered if the thrombocyte count is approximately $75\,000/\text{mm}^3$, halved if it is in the range of $50\,000$ to $75\,000/\text{mm}^3$, and discontinued less than $50\,000/\text{mm}^3$. If the thrombocyte count is low, CD3+ cell counts can help to inform decisions on rATG dose changes or discontinuation. Lymphocyte and neutrophil counts must also be taken into account and can trigger dose reductions or withdrawal (Table 4).

Assessment of an effect on thrombocyte count can, of course, be complicated by thrombocyte transfusions. Similarly, the hematological effects of mycophenolate mofetil therapy also need to be taken into account.

Premedication

Each rATG infusion should be preceded by concomitant therapy to minimize short-term adverse events, such as fever and rash, for example, a combination of H₁ and H₂ blockers plus intravenous steroids, with antipyretic therapy to prevent fever.

TABLE 4.

Hematological triggers to adjust or discontinue rATG administration

| Parameter | Count | Action |
|--|-----------------------|---|
| Platelets, $/\text{mm}^3$ ^a | $>75\,000$ | No change |
| | $\sim 75\,000$ | Reduce dose |
| | $50\,000$ - $70\,000$ | Half dose |
| Leukocytes, $/\text{mm}^3$ | $<50\,000$ | Discontinue |
| | >3000 | No action at high levels but consider dose reduction if 3000 - 5000 |
| Neutrophils, $/\text{mm}^3$ | 2000 - 3000 | Consider half dose |
| | <2000 | Discontinue |
| Lymphocytes, $/\text{mm}^3$ | >1000 | No action |
| | <1000 | Discontinue or interrupt for 1 day and reassess ^b |
| Lymphocytes, $/\text{mm}^3$ | <100 | Interrupt for 1 d and reassess |

^a Effect of thrombocyte transfusions should be taken into account. If platelet count is high but bleeding occurs, consider pausing rATG administration.

^b Due to risk of fungal infections.

rATG Dosing in Children

In contrast to adult recipients, registry data show that the majority of pediatric heart transplant patients receive induction therapy: a rate of 71% was recorded in the 2013 International Society for Heart and Lung Transplantation Pediatric Heart Transplant Report.⁵⁴ One reason is the widespread practice of gradually weaning children off oral steroids to avoid a negative impact on growth and pubertal development, in addition to the other side effects of steroids.⁵⁵ Induction therapy with rATG is universal in ABO-incompatible heart transplantation in children,⁵⁶ but cases are rare. Overall, experience and published data concerning the use of rATG in pediatric patients are limited due to the low numbers worldwide. Most pediatric heart transplant programs perform fewer than 5 transplants a year; of 172 centers reporting data to the ISHLT registry in 2013, only 45 transplanted 5 or more heart grafts in children.⁵⁷

Only retrospective data are available concerning the use of rATG in children undergoing heart transplantation (Table 5). Total doses administered have ranged from a mean of 5.7 mg/kg⁵⁸ to a median of 8 mg/kg,⁴⁷ although 1 early study included doses of 17.5 mg/kg.⁵⁹ Although the use of OKT3 induction has been linked with an increased risk for PTLD,⁶⁰ the relevance of rATG dosing in children remains a matter for debate (see also *Impact of rATG dose on safety*). The limited published evidence indicates that a maximum dose of 7.5 mg/kg is adequate in standard risk children undergoing heart transplantation with CNI-based maintenance therapy.^{48,58}

The authors adopt a similar approach to rATG dosing for children to that used in adults. Dosing is tailored according to whether patients are low risk or high risk (eg, pretransplant donor-specific antibody, prior cardiac surgery, retransplantation, MCS before transplant). The duration of rATG infusion should be not less than 6 hours. The total dose can be as low as 3.5 mg/kg but should not exceed 7.5 mg/kg, and use of high rATG dosages should be avoided based on a potential risk for PTLD. The hematological triggers for dose modification or discontinuation used in adults also apply to children (Table 4).

The role of rATG in neonatal heart transplantation needs further studies and investigation.

TABLE 5.**Studies of rATG induction and CNi therapy in pediatric heart transplant recipients**

| Study | Design/Follow-Up | Population | n | rATG dose and duration | Total rATG dose | CNi regimen | Adjunctive therapy | Outcome | Comment |
|--|--|--|----|--|------------------|-------------------------------|--|--|--|
| Singh et al, 2010 ⁴⁸ | Retrospective single center; median, 19 mo | Children with PRA <10%, negative donor-specific cross match on flow cytometry ^a | 55 | 1.5 mg/kg × 5 | 7.5 mg/kg | TAC started days 2-3 | MMF started after day 5, IV steroids (median, 5 d) | 13% rejection at 1 y (3 grade 2R, 5 AMR by last follow-up) | 80% of patients remained steroid-free at last follow-up ^b |
| Pollock-BarZiv et al, 2008 ⁵⁸ | Retrospective single center; mean, 2.3 y | Unselected population <18 y including 36.4% HLA sensitized patients | 55 | 1.5 mg/kg per day via 24-h infusion; ^{c, d} mean, 3.8 d | Mean, 5.7 mg/kg | Immediate TAC or CsA | MMF or AZA, steroids | 51% BPAR grade ≥2R | No infectious deaths |
| Di Filippo et al, 2003 ⁴⁷ | Retrospective 2 centers; median, 6.3 y | All rATG-treated patients <18 years | 30 | 1-2 mg/kg × 5 doses ^c | Median, 8 mg/kg | CsA started from day 2 | AZA, steroids | 50% acute rejection in year 1 | Shorter time to CMV infection with higher doses of rATG (P = 0.002) |
| Parisi et al, 2003 ⁵⁹ | Retrospective single center; mean, 3 y | Consecutive rATG-treated patients <18 y | 31 | 1-2.5 mg/kg for 1-7 d (age-dependent dosing) ^c | Up to 17.5 mg/kg | CsA (start day not specified) | ± MMF or AZA, ± Steroids | 9.7% BPAR 3A, 32.3% BPAR grade 1A | 61.3% steroid-free at last follow-up |

^a 6 patients with PRA ≥ 10% were included.^b That is, 40/60 patients who were alive at discharge.^c Adjusted according to predefined hematological criteria.^d Started intraoperatively.

AMR, antibody-mediated rejection; IV, intravenous; PRA, panel reactive antibody.

Proposed Early CNi Dosing Strategy in Adults Receiving rATG Induction**Timing of CNi Introduction**

The timing of CNi start and the initial dose is driven by the patient's renal function in the immediate posttransplant period. Although no firm threshold for impaired renal function exists, an estimated glomerular filtration rate value below 50 to 60 mL/min per 1.73 m² appears to be a relevant cutoff. In our previous proposal for use of rATG after heart transplantation, patients with impaired renal function were categorized by cardiorenal syndrome type 1 or 2 (kidney dysfunction arising from cardiac causes) or type 3 or 4 (primary kidney disease).⁶¹ We recognize that it can be difficult to distinguish between cardiorenal categories at the time of transplant unless renal disease has been diagnosed previously (eg, diabetic nephropathy or glomerulonephritis). Therefore, decision-making is frequently empiric, based on the rate and extent of renal function improvement. Where there is a steady and marked reduction in serum creatinine in the first 1 to 2 days posttransplant, CNi can be started from day 3 onward. If creatinine levels decline more slowly compared with baseline, this suggests the presence of chronic kidney disease unrelated to cardiac function. Here, the start of CNi can be delayed until between day 5 and day 10 (but no later), with less aggressive initial CNi trough concentrations.

Defining CNi Exposure

For patients at low or standard immunological risk who have poor renal function, CNi starting doses and targets should be reduced. A trough concentration target range of 6 to 10 ng/mL for tacrolimus, and 100 to 200 ng/mL for CsA, appears appropriate (Figure 2A). Close monitoring for clinical or histological signs of rejection is essential to support individualized dosing with the aim of keeping CNi exposure as low as possible compatible with prevention of rejection. Longer-term results from the SCHEDULE study indicate that switching from CNi therapy to everolimus at 7 to 11 weeks posttransplant can improve preservation of renal function.⁵⁰

For patients at high immunological risk, tacrolimus is generally preferable to CsA, with higher target levels (tacrolimus, 10-15 ng/mL or CsA, 200-300 ng/mL if used) (Figure 2B). Calcineurin inhibitor therapy should be started by day 3, and dosing should be adjusted more aggressively than in low-risk patients, to achieve target exposure rapidly. Subsequent tapering of CNi exposure is dependent on confirmed absence of rejection or adequate immunological activity. In high-risk patients, avoiding rejection takes precedence over protection of renal function.

A substantial minority of patients before transplant are on MCS with both infection and impaired renal function. This presents the daunting challenge of balancing the need to prevent rejection without exacerbating infection, and as a somewhat lower priority minimizing early CNi-related nephrotoxicity. Calcineurin inhibitor exposure must be reduced due to the presence of infection and is likely to resemble the exposure targets used for low-risk individuals with no infection, that is, tacrolimus 6 to 10 ng/mL and CsA 100 to 200 ng/mL (Figure 2C). Infection should be monitored closely, and CNi exposure can be increased once the infection clears. The pretransplant anti-infective

TABLE 6.
Proposals for randomized studies of rATG with CNi therapy in heart transplantation

| Objective | Population | Comparator groups | Key endpoints |
|--|--|--|---|
| Assess whether rATG + delayed CNi improves renal function preservation | Poor renal function, eg, serum creatinine > 132 $\mu\text{mol/L}$, ^a unselected for immunological risk | rATG + delayed CNi, no induction + immediate CNi | Renal function, need for renal replacement therapy, rejection, rATG-related toxicity, infection |
| Determine optimal rATG dose | Low/standard risk population (including subpopulation analysis with/without VAD) | Cumulative rATG dose 4.5 vs 6.0 vs 7.5 mg/kg | Rejection, infections, other rATG-related toxicity |
| Define timing of rATG initiation | Unselected | Preoperative vs postoperative initiation, eg, 6.0 mg/kg total dose | Primary graft function, thrombocytopenia, and bleeding |
| Compare fixed rATG dosing vs immune-guided rATG dosing | Unselected | Fixed dose, eg, 1.5 mg/kg for 3 d vs dosing adjusted according to CD3 ⁺ or lymphocyte count | Cumulative rATG dose, rejection, infections, other rATG-related toxicity |

^aRenal function should be assessed at time of listing for transplant and updated at each subsequent evaluation
VAD, ventricular assist device.

regimen should be continued for 10 to 14 days after transplant, and any interactions between the anti-infectives and immunosuppressive agents should be identified and taken into account.

Overlapping of rATG and CNi Administration

In patients at low or standard immunological risk, the final rATG dose should be completed before the first dose of CNi, that is, no overlap. Generally, there should be no gap between the 2 therapies, for example, last rATG dose started in the morning with the first CNi dose given in the evening. For patients at high immunological risk, an overlap of up to 3 to 4 days between the end of the last rATG infusion and the first CNi dose can be considered.

CONCLUSIONS

After our previous proposals regarding patient selection for rATG induction after heart transplantation, we have sought here to provide guidance on dosing protocols in particular clinical settings. It is not possible to provide firm recommendations regarding rATG dosing or the optimal timing, type, and extent of CNi exposure due to the profound shortage of well-designed clinical trials in this area. The proposals presented here represent the authors' best judgement according to the available literature and personal experience. There is a clear need for more studies, and although inevitably limited by the number of available patients, we have proposed trials which we consider to be the most urgent to define optimal treatment protocols for rATG with CNi therapy after heart transplantation (Table 6).

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