Clinical Study

The Effect of Combination Therapy with Rituximab and Intravenous Immunoglobulin on the Progression of Chronic Antibody Mediated Rejection in Renal Transplant Recipients

Gun Hee An, Jintak Yun, Yu Ah Hong, Marina Khvan, Byung Ha Chung, Bum Soon Choi, Cheol Whee Park, Yeong Jin Choi, Yong-Soo Kim, and Chul Woo Yang

1 Division of Nephrology, Department of Internal Medicine, Seoul St. Mary’s Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea
2 Division of Nephrology, Department of Internal Medicine, Korea University Guro Hospital, Seoul, Republic of Korea
3 Dialysis Department, National Research Center for Maternal and Child Health, Astana, Kazakhstan
4 Transplant Research Center, Seoul St. Mary’s Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea
5 Department of Hospital Pathology, Seoul St. Mary’s Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea

Correspondence should be addressed to Chul Woo Yang; yangch@catholic.ac.kr

Received 15 June 2013; Revised 28 October 2013; Accepted 11 November 2013; Published 29 January 2014

Academic Editor: Qiuyan Sun

Copyright © 2014 Gun Hee An et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The treatment for chronic active antibody-mediated rejection (CAMR) remains controversial. We investigated the efficacy of rituximab (RTX) and intravenous immunoglobulin (IVIg) for CAMR. Eighteen patients with CAMR were treated with RTX (375 mg/m²) and IVIg (0.4 g/kg) for 4 days. The efficacy of RTX/IVIg combination therapy (RIT) was assessed by decline in estimated glomerular filtration rate per month (ΔeGFR) before and after RIT. Patients were divided into responder and nonresponder groups based on decrease and no decrease in ΔeGFR, respectively, and their clinical and histological characteristics were compared. Response rate to RIT was 66.7% (12/18), and overall ΔeGFR decreased significantly to 0.4 ± 1.7 mL·min⁻¹·1.73 m² per month 6 months after RIT compared to that observed 6 months before RIT (1.8 ± 1.0, \( P < 0.05 \)). Clinical and histological features between the 12 responders and the 6 nonresponders were not significantly different, but nonresponders had a significantly higher proteinuria level at the time of RIT (2.5 ± 2.5 versus 7.0 ± 3.5 protein/creatinine (g/g), \( P < 0.001 \)). The effect of the RIT on ΔeGFR had dissipated in all patients by 1 year post-RIT. Thus, RIT delayed CAMR progression, and baseline proteinuria level was a prognostic factor for response to RIT.

1. Introduction

Circulating alloantibodies are found in a substantial number of renal allograft recipients, and the presence of these alloantibodies is significantly correlated with the development of allograft injury and later graft loss [1–3]. In renal allograft tissue, chronic injury is represented microscopically as transplant glomerulopathy and diffuse C4d deposition in peritubular capillaries (PTCs); recently, it was included as new disease entity named chronic antibody-mediated rejection (CAMR) in the update of the Banff 05 classification [4]. Usually the prognosis of CAMR is poor, and conventional immunosuppressants mainly targeting T cell-mediated immunity cannot prevent or reverse it [5–7]. Therefore, some researchers have suggested that therapies directed at the humoral response may be required for the treatment of CAMR [3].

Recently, some reports have suggested that the combined use of rituximab (RTX) and intravenous immunoglobulin (IVIg) therapy may be useful for the treatment of CAMR. Billing et al. published their experience with the RTX and IVIg combination protocol for treatment of CAMR in 6
pediatric patients, and they subsequently reported the long-
term effects of this protocol [8, 9]. In adult renal transplant
recipients, only a few studies have been published. Fehr et al.
demonstrated that allograft function of CAMR was improved
or stabilized with the RTX and IVIg combination therapy in
4 cases [10]. Our preliminary study also showed that the
combination therapy was effective in delaying the progression
of CAMR, especially in its early stages [11]. However, the
above studies were conducted with small numbers of adult
patients during periods of relatively short duration.

For these reasons, we decided to perform a study inves-
tigating the efficacy of the RTX and IVIg protocol for the
treatment of CAMR, using a larger group of adult patients
and with a longer period of followup.

2. Patients and Method

2.1. Diagnosis of CAMR. The diagnosis of CAMR was based
on the update on Banff classification: (1) transplant glomeru-
lopathy and severe peritubular capillary basement membrane
multilayering (PTCBMM), interstitial fibrosis (IF) and tubu-
lar atrophy (TA) with or without peritubular capillary loss,
and fibrous intimal thickening in arteries without internal
elastic duplication; (2) diffuse C4d deposition in PTCs; and
(3) presence of donor-specific anti-HLA antibody (DSA) [4].
Among allograft biopsies done between September 2009 and
December 2012, in Seoul St. Mary’s Hospital, 16 cases met
the above Banff criteria. We also included 2 patients who
did not fully satisfy with the criteria (negative HLA-DSA
and C4d score 0 and score 1) but showed typical transplant
glomerulopathy with slowly deteriorating graft function.
Finally 18 patients were included in this study.

2.2. Patient Characteristics. Patient characteristics are shown
in Table 1. The mean age of the patients was 44.0 ± 7.1 years
at the time of CAMR diagnosis; 13 patients (72%) were male.
Of the 18 patients, 11 (61%) received kidneys from living
donors and 2 patients had histories of retransplantation.
Eight of the 18 patients (44%) experienced acute rejec-
tion, including both antibody-mediated and T cell-mediated
rejections, before CAMR. The median time posttransplant
until the diagnosis of CAMR by renal graft biopsy was
93.2 months (range: 8.2–214.9). The follow-up duration after
treatment was 14.1 months (range: 1.4–31.9). This study was
approved by the Institutional Review Board of our institution
(KC12RISI0070).

2.3. Protocol of Rituximab/IVIg Combination Therapy for
CAMR. The protocol in our institution for the treatment of
CAMR has been described previously (RIT protocol) [11].
Briefly, all patients were treated with IV RTX (375 mg/m^2
once on day 1 followed by IVIg, 0.4 g/kg, once daily for 4 days.
Pulse methylprednisolone at a dose of 500 mg IV was admin-
istered daily for the first 3 days, followed by oral prednisolone,
tapered to 30 mg/day. We measured anti-HLA antibody using
Luminex solid-phase assays (LSA; Tepnel Life codes Corp.,
Stamford, CT) at the time of biopsy. If the type of anti-HLA
antibody detected in the patient corresponded to the HLA
type of the donor, it was regarded as a donor-specific anti-
HLA antibody (HLA-DSA). The results were presented as 4
levels, according to the median fluorescent intensity (MFI)
value: strong, >10,000; moderate, 5000–10,000; weak, 1000–
5000; and negative, <1000.

2.4. Efficacy of Treatment Protocol. The primary outcome
of this study was improvement in allograft function after
treatment. Allograft function was assessed on the basis of
serum creatinine levels and estimated glomerular filtration
rate (eGFR), using the modification of the diet in renal disease
(MDRD) formula (eGFR = 186.3 × serum creatinine^{-1.154} ×
age^{-0.263} × [0.742 if female] mL/min\1.73 m^2) [12]. We
calculated the decline in the rate of eGFR per month (ΔeGFR)
during the 6 months before and after RIT and at 6-month
intervals until the last followup. We also evaluated the amount
of proteinuria (g protein/g creatinine (g/g)) in random urine
chemistry, collected 6 months before RIT, at the time of RIT.

| Table 1: Baseline characteristics of patients populations at treatment of CAMR. |
|-------------------------------------------------|
| Clinical parameters                            | All patients (n = 18) |
|------------------------------------------------|
| Age (years)                                    | 44.0 ± 7.1           |
| Male gender, n (%)                             | 13 (72)              |
| BMI (Kg/m^2)                                   | 24.3 ± 2.7           |
| Primary renal disease                          |                       |
| cGN, n (%)                                     | 7 (39)               |
| HBP, n (%)                                     | 6 (33)               |
| DM, n (%)                                      | 1 (6)                |
| Unknown, n (%)                                 | 4 (22)               |
| Dialysis type before KT                        |                       |
| Hemodialysis, n (%)                            | 13 (72)              |
| Peritoneal dialysis, n (%)                     | 5 (28)               |
| Dialysis duration, month                       | 24.6 ± 24.5          |
| Donor type, Living, n (%)                      | 11 (61)              |
| Multitransplant History, n (%)                 | 2 (11)               |
| Main immunosuppressant                         |                       |
| Cyclosporine, n (%)                            | 6 (33)               |
| Tacrolimus, n (%)                              | 12 (67)              |
| Previous acute rejection, n (%)                | 8 (44)               |
| Serum Cr (mg/dL)                               | 2.3 ± 0.9            |
| MDRD eGFR (mL/min/1.73 m^2)                    | 35.8 ± 16.1          |
| Proteinuria (g/day)                            | 4.3 ± 3.6            |
| Time posttransplant until diagnosis, month     | 93.2 ± 61.5          |
| Time posttreatment, month                      | 14.1 ± 9.3           |
| HLA mismatch number                            | 3.2 ± 1.4            |
| HLA-DSA                                         |
| Not done, n (%)                                | 2 (11)               |
| Positive, Class I, n (%)                       | 2 (11)               |
| Positive, Class II, n (%)                      | 5 (28)               |
| Negative, n (%)                                | 9 (50)               |

CAMR: chronic antibody mediate rejection; BMI: body mass index; cGN: chronic glomerulonephritis; Cr: creatinine; MDRD eGFR: estimated GFR using the Modification of Diet in Renal Disease Study equation; HLA-DSA: donor specific anti-HLA antibody.
3.1. Histologic and Immunologic Characteristics. Table 2 shows the histological characteristics at diagnosis of CAMR. Transplant glomerulopathy and PTCBMM were found in 10 patients (59%). Advanced chronic changes such as interstitial fibrosis and tubular atrophy were detected in most patients (17/18, 94%), and the staining for C4d was diffusely positive in 14 out of 18 patients (82%). In 16 patients who were examined for HLA-DSA using LSA at the time of biopsy, anti-HLA antibody was detected in 10 patients, and of these, 6 patients were identified to have HLA-DSA. HLA-DSA showed strong MFI in only 1 patient, moderate intensity in 3, and weak intensity in 2.

3.2. The Response to RTX/IVIG Treatment in Terms of Allograft Function. All patients tolerated RIT well and completed treatment without immediate adverse effects. Before RIT, progressive deterioration of allograft function was found in all patients. At 6 months before RIT, eGFR was 48.1 ± 17.5 mL·min⁻¹·1.73 m⁻² and progressively declined to 37.1 ± 15.6 mL·min⁻¹·1.73 m⁻² at the time of RIT (P < 0.001). The calculated ΔeGFR was 1.8 ± 1.0 mL·min⁻¹·1.73 m⁻² per month during that period. Six months after RIT, eGFR was 34.7 ± 19.2 mL·min⁻¹·1.73 m⁻², which is similar to that at the time of RIT (P = 0.40), and ΔeGFR 6 months after RIT was 0.4 ± 1.7 mL·min⁻¹·1.73 m⁻² per month, which is significantly lower than that at 6 months before RIT (P < 0.05). Proteinuria increased significantly from 3.0 ± 3.7 g/g at 6 months before RIT to 4.3 ± 3.6 g/g at the time of RIT (P < 0.05). The amount of proteinuria showed a decreasing trend at 6 months since RIT (3.0 ± 2.2 g/g, versus that at the time of RIT, P = 0.129) compared to the value at the time of RIT; this trend was observed even at the last followup (2.9 ± 2.7 g/g, versus at the time of RIT, P = 0.136).

3.3. Comparison between Responder and Nonresponder Groups. According to the change in ΔeGFR during 6 months after RIT compared to that observed 6 months before RIT, 12 patients (67%) met the criteria for the responder group, and the other 6 patients, for the nonresponder group. The eGFR at 6 months before RIT (45.4 ± 16.4 versus 54.5 ± 20.2 mL·min⁻¹·1.73 m⁻², P = 0.347) and that at the time of RIT (34.2 ± 14.3 versus 39.0 ± 20.3 mL·min⁻¹·1.73 m⁻², P = 0.568) were not significantly different between 2 groups. The ΔeGFR (1.9 ± 1.1 versus 1.8 ± 0.9 mL·min⁻¹·1.73 m⁻² per month, P = 0.83) 6 months before RIT did not differ between the 2 groups, as well. ΔeGFR decreased to −0.3 ± 1.2 mL·min⁻¹·1.73 m⁻² per month 6 months after RIT in the responder group compared to that observed 6 months before RIT (1.9 ± 1.1 mL·min⁻¹·1.73 m⁻² per month, P < 0.01). In contrast, nonresponders showed relatively higher ΔeGFRs 6 months after RIT (2.5±0.8 mL·min⁻¹·1.73 m⁻² per month) compared to that before 6 months, which suggests that the allograft function was still rapidly deteriorating (P = 0.105; Figure 1). In comparison, the amount of proteinuria at the time of RIT was significantly higher in the nonresponder group (7.0 ± 3.5 g/g) than in the responder group (2.8 ± 2.8 g/g, P < 0.05). However, the histological features and other clinical parameters did not show any significant differences. The positivity of HLA-DSA at biopsy did not differ either (Table 3).

### Table 2: Histopathology of allograft biopsy and grading according to Banff 05

| Characteristics (total n = 18) | Score | N (%) |
|-------------------------------|------|-------|
| Transplant glomerulopathy (cg) | 0    | 7 (41) |
|                               | 1    | 0 (0)  |
|                               | 2    | 0 (0)  |
|                               | 3    | 10 (59) |
| PTC BMM                      | (-) | 7 (41) |
|                              | (+) | 10 (59) |
| C4d in PTC                   | 0   | 3 (18) |
|                              | 1   | 3 (18) |
|                              | 2   | 9 (53) |
|                              | 3   | 2 (11) |
| Peritubular capillaritis (ptc)| 0   | 4 (24) |
|                               | 1   | 1 (6)  |
|                               | 2   | 5 (29) |
|                               | 3   | 7 (41) |
| Interstitial fibrosis (ci)/Tubular atrophy (ct)| 0 | 1 (6) |
|                                | 1 | 9 (50) |
|                                | 2 | 7 (38) |
|                                | 3 | 1 (6)  |

PTC: peritubular capillary; BMM: basement membrane multilayering.

*17 subjects had available data about transplant glomerulopathy, PTC BMM and C4d in PTC.

### 2.5. Statistical Analysis. Data were expressed as mean and standard deviation (SD). Means of normally distributed data were compared using Student's t-test; a χ²-test was used to compare proportions. The changes in eGFR before and after treatment were evaluated by paired comparison. Graft survival rates after RIT were calculated using Kaplan-Meier analysis, and we used the log-rank analysis to compare survival rates between groups. The association of the degree of histological lesions with the response to RIT was explored with Fisher’s exact test. In all analyses, P < 0.05 (two-tailed) was taken to indicate statistical significance.

### 3. Results

#### 3.1. Histologic and Immunologic Characteristics. Table 2 shows the histological characteristics at diagnosis of CAMR. Transplant glomerulopathy and PTCBMM were found in 10 patients (59%). Advanced chronic changes such as interstitial fibrosis and tubular atrophy were detected in most patients (17/18, 94%), and the staining for C4d was diffusely positive in 14 out of 18 patients (82%). In 16 patients who were examined for HLA-DSA using LSA at the time of biopsy, anti-HLA antibody was detected in 10 patients, and of these, 6 patients were identified to have HLA-DSA. HLA-DSA showed strong MFI in only 1 patient, moderate intensity in 3, and weak intensity in 2.
### Table 3: Comparison of parameters between responder and nonresponder groups at treatment of CAMR.

| Clinical parameters                      | Responder (n = 12) | Nonresponder (n = 6) | P value |
|------------------------------------------|--------------------|----------------------|---------|
| Age (years)                              | 44.0 ± 7.0         | 44.3 ± 7.9           | 0.928   |
| Male gender, n (%)                       | 8 (67)             | 5 (83)               | 0.615   |
| BMI (Kg/m²)                              | 24.0 ± 3.2         | 24.9 ± 1.8           | 0.518   |
| Multitransplant History, n (%)           | 1.0 ± 0.0          | 1.3 ± 0.5            | 0.175   |
| Previous acute rejection, n (%)          | 0.5 ± 0.7          | 0.7 ± 0.8            | 0.650   |
| Serum Cr (mg/dL)                         | 2.3 ± 0.7          | 2.4 ± 1.3            | 0.809   |
| MDRD eGFR (mL/min/1.73 m²)               | 34.2 ± 14.3        | 39.0 ± 20.3          | 0.568   |
| Proteinuria (g/day)                      | 2.8 ± 2.8          | 7.0 ± 3.5            | 0.015   |
| Time posttransplant before CAMR diagnosis, month | 106.1 ± 65.6 | 67.3 ± 46.8          | 0.217   |
| Time posttreatment, month                | 13.9 ± 7.8         | 14.6 ± 12.6          | 0.889   |
| HLA-DSA, MFI *                           |                    | 0.629                |         |
| Strong, n (%)                            | 1 (10)             | 0 (0)                |         |
| Moderate, n (%)                          | 1 (10)             | 2 (50)               |         |
| Weak, n (%)                              | 2 (20)             | 0 (0)                |         |
| Negative, n (%)                          | 6 (60)             | 2 (50)               |         |
| Histologic parameters                    |                    |                      |         |
| Transplant glomerulopathy                | 1.75 ± 1.5         | 1.8 ± 1.6            | 0.953   |
| PTCBMM (+ /−)                            | 7/5                | 3/2                  | 0.951   |
| Peritubular capillaritis                 | 1.9 ± 1.3          | 1.8 ± 1.1            | 0.864   |
| IF/TA                                    | 1.4 ± 0.8          | 1.5 ± 0.5            | 0.821   |
| C4d in PTC                               | 2.25 ± 1.4         | 2.8 ± 0.8            | 0.436   |

CAMR: chronic antibody mediate rejection; BMI: body mass index; Cr: creatinine; MDRD eGFR: estimated GFR using the Modification of Diet in Renal Disease Study equation; HLA-DSA: donor specific anti-HLA antibody; PTC: peritubular capillary; BMM: basement membrane multilayering.

* 16 out of 18 subjects take HLA-DSA and 14 had available data.

3.4. The Clinical Outcome during Long-Term Followup after Treatment. During long-term followup, only 1 case developed herpes zoster infection; no other serious complications were detected. Four patients (39%) exclusively in the nonresponder group experienced allograft loss at 1.4, 5.1, 8.6, and 11.9 months since treatment with RIT, and no allograft loss was noted in the responder group (Figure 2). In 7 patients with a follow-up duration of >12 months, the ΔeGFR observed 6–12 months after RIT (0.5 ± 0.7 mL·min⁻¹·1.73 m² per month) was still lower than that observed 6 months before RIT (1.6 ± 1.1 mL·min⁻¹·1.73 m² per month, P < 0.05). However, ΔeGFR showed an increasing trend over the final 12 months until the last followup (1.2 ± 0.8 mL·min⁻¹·1.73 m² per month), at which it showed a value similar to that 6 months before RIT (Figure 3).

4. Discussion

In this study, 18 adult patients who were diagnosed as CAMR or suspicious of CAMR were treated with RTX and IVIg combination protocol. After this combination treatment, the rate of decline in allograft function decreased significantly in most patients, which suggests that this combination therapy is effective in delaying the progression of CAMR.

The effect of the combination therapy with RTX and IVIg on CAMR in pediatric patients has been reported in previous studies [8, 9]. However, the effect of the combination therapy in adult renal transplant recipients has not been established. We previously reported the beneficial effect of that therapy in 6 adult patients [11]. In this study, we investigated the effect of our protocol in larger patient group with longer follow-up period. The detailed mechanism for the development of CAMR has not been fully elucidated; however, in nature, antibody-mediated injury may be the main pathogenetic mechanism of CAMR [2, 3]. IVIg can suppress immunoglobulin synthesis, has anti-idiotypic activity against DSA (with resultant neutralization of DSA), blocks the Fc receptor, inhibits complement activation, and has anticytokine activity [13]. RTX, a chimeric anti-CD20 monoclonal antibody, can induce antibody-dependent cytotoxicity, complement-dependent cell killing, and apoptotic cell death, especially in B cells. Consequently RTX depletes B cells and interferes with antigen-presenting cell activity of B cells [14]. For this reason, RTX and IVIg, which target humoral immunity by different action mechanism, have been proposed as a therapeutic option for CAMR [8].

At first, we investigated the effect of this combination therapy on the progression of CAMR by comparing the rate of decline in eGFR before and after RIT. After RIT, the overall ΔeGFR slopped down, and in particular, in 12 out of 18 patients (67%), the ΔeGFR showed a significant decrease, which is similar to the result from a previous report [9]. The amount of proteinuria, which is poor prognostic factor for allograft outcome, showed a decrease after RIT, as well [15–17]. In addition, this protocol is well tolerated, and fatal
infectious complications were not detected during the long-term follow-up period. All the above findings suggest that this protocol is not only effective but also safe for treating patients with CAMR.

However, 6 patients did not show a significant response to therapy and 4 out of the 6 patients in the nonresponder group experienced allograft failure within 1 year since treatment with RIT. To investigate the risk factors associated with this lack of response to the RIT protocol, we compared the clinical parameters between the responder and nonresponder groups. We did not find any significant differences in clinical characteristics. Of note, however, the amount of proteinuria at the time of RIT was significantly higher in nonresponder group than in the responder group. This finding is consistent with a previous study that showed that proteinuria is associated with more severe acute and chronic allograft rejection [18]. In contrast, allograft function at the time of RIT and the rate of decline in eGFR observed 6 months before RIT did not differ between the two groups. This suggests that the severity of allograft dysfunction does not predict the response to treatment.

In contrast to some previous reports, histological features were not associated with clinical outcomes in our study. For example, the proportion of transplant glomerulopathy and the severity of IF/TA, which is an important morphologic pattern of chronic kidney allograft injury, did not differ between the two groups [5, 19–21]. This result suggests that the histologic pattern is a prognostic factor in CAMR that progresses without intervention; however, it may not be an accurate prognostic indicator with the use of antihumoral therapy. Indeed, a previous study reported that pathological correlations that predicted the response to therapy were not identified [22]. Further investigation may be required to clarify this issue.

In the long-term followup, the therapeutic effect of RIT showed a decreasing trend with time, especially after 1 year since RIT initiation. In this study, 4 patients with a follow-up duration >2 years were included, and the time-dependent decrease in eGFR was detected. Interestingly, this pattern was found 6 months after RIT treatment not only in the nonresponder group but also in the responder group. The decrease may be associated with the duration of the B cell-depleting effect of RTX. A previous study showing RTX-induced B cell depletion in the peripheral blood indicated that patients recover approximately 6 months since RTX infusion [23], which suggests that the therapeutic effect of RIT on the progression of CAMR may be limited to this time period; accordingly, repeated RIT therapy or other additional strategies for humoral immunity such as bortezomib may be necessary to prolong the therapeutic effect [24–27].
observed 6 months before RIT. At every 6-month interval and the followup over 12 months. With time, Figure 3: Changes in
morphologic evidence of antibody-mediated rejection [30].
or alloantibody is not detected even in the presence of allograft function. Second, there is a possibility that C4d on allograft biopsy is responsible for slowly deteriorating is strongly suggested that typical transplant glomerulopathy detected. We enrolled those patients for two reasons. First, it is not satisfied with the diagnostic criteria of CAMR; they did not make a clear-cut diagnosis.

The combination of RTX and IVIg showed a relatively long-term effect in pediatric renal transplant recipients with CAMR over 2 years, in contrast to this study [8, 9]. The possible reason is that the response to RIT may differ between adult and pediatric CAMR patients. The hematopoietic bone marrow contains mostly naive B cells of diverse specificities and has only a small number of memory B cell clones in childhood. Usually, memory B cells and plasma cell, which are responsible for the development of CAMR, accumulate with age [28]. Hence, these different immunologic characteristics, the higher memory B cell, and plasma cell pool in adult patients, may be associated with the limited long-term effect to RIT [29].

In our study, we included two patients who were not satisfied with the diagnostic criteria of CAMR; they did not show C4d deposition on biopsy tissue and DSA was not detected. We enrolled those patients for two reasons. First, it is strongly suggested that typical transplant glomerulopathy on allograft biopsy is responsible for slowly deteriorating allograft function. Second, there is a possibility that C4d or alloantibody is not detected even in the presence of morphologic evidence of antibody-mediated rejection [30]. It suggests that CAMR is a dynamic process and is difficult to make a clear-cut diagnosis.

This study has some limitations. First, we did not perform follow-up biopsies. Despite a significant decrease in \( \Delta eGFR \), we could not prove this benefit in the allograft tissue, for example, in the reduction of positive C4d or transplant glomerulopathy. Second, we did not include an untreated control group with CAMR. A larger randomized study, including treated subjects and untreated controls, may be required to prove the efficacy of RIT.

In conclusion, this study showed that the combination of RTX and IVIg is an effective treatment in delaying the progression of CAMR. In addition, the amount of proteinuria at the time of treatment is the most important prognostic factor for predicting the patient’s response to RTX/IVIG combination therapy. However, the therapeutic effect showed a decreasing pattern over 1 year after RIT, which indicates that additional therapeutic strategy may be required in such patients.

**Conflict of Interests**
The authors declare that there is no conflict of interests.

**Acknowledgment**
This study was supported by a grant of the Korean Health Technology R&D Project, Ministry for Health & Welfare, Republic of Korea (HI09C1555).

**References**

[1] T. Hirai, N. Kohei, K. Omoto, H. Ishida, and K. Tanabe, “Significance of low-level DSA detected by solid-phase assay in association with acute and chronic antibody-mediated rejection,” Transplant International, vol. 25, no. 9, pp. 925–934, 2012.

[2] J. Pascual, M. J. Perez-Saez, M. Mir, and M. Crespo, “Chronic renal allograft injury: early detection, accurate diagnosis and management,” Transplantation Reviews, vol. 26, no. 4, pp. 280–290, 2012.

[3] G. Einecke, B. Sis, J. Reeve et al., “Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure,” The American Journal of Transplantation, vol. 9, no. 11, pp. 2520–2531, 2009.

[4] K. Solc, R. B. Colvin, L. C. Racusen et al., “Banff 05 meeting report: differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ("CAN"),” The American Journal of Transplantation, vol. 7, no. 3, pp. 518–526, 2007.

[5] F. G. Cosio, J. M. Gloor, S. Sethi, and M. D. Stegall, “Transplant glomerulopathy,” The American Journal of Transplantation, vol. 8, no. 3, pp. 492–496, 2008.

[6] S. Mauiyedi, P. D. Pelle, S. Saimon et al., “Chronic humoral rejection: identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries,” Journal of the American Society of Nephrology, vol. 12, no. 3, pp. 574–582, 2001.

[7] P. I. Terasaki and M. Ozawa, “Predictive value of HLA antibodies and serum creatinine in chronic rejection: results of a 2-year prospective trial,” Transplantation, vol. 80, no. 9, pp. 1194–1197, 2005.

[8] H. Billing, S. Rieger, J. Ovens et al., “Successful treatment of chronic antibody-mediated rejection with IVIG and rituximab in pediatric renal transplant recipients,” Transplantation, vol. 86, no. 9, pp. 1214–1221, 2008.
[9] H. Billing, S. Rieger, C. Susal et al., “IVIG and rituximab for treatment of chronic antibody-mediated rejection: a prospective study in paediatric renal transplantation with a 2-year follow-up,” Transplant International, vol. 25, no. 11, pp. 1165–1173, 2012.

[10] T. Fehr, B. Rüsi, A. Fischer, H. Hopfer, R. P. Wüthrich, and A. Gaspert, “Rituximab and intravenous immunoglobulin treatment of chronic antibody-mediated kidney allograft rejection,” Transplantation, vol. 87, no. 12, pp. 1837–1841, 2009.

[11] Y. A. Hong, H. G. Kim, S. R. Choi et al., “Effectiveness of rituximab and intravenous immunoglobulin therapy in renal transplant recipients with chronic active antibody-mediated rejection,” Transplantation Proceedings, vol. 44, no. 1, pp. 182–184, 2012.

[12] A. S. Levey, J. Coresh, T. Greene et al., “Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate,” Annals of Internal Medicine, vol. 145, no. 4, pp. 247–254, 2006.

[13] S. C. Jordan, A. A. Vo, A. Peng, M. Toyoda, and D. Tyan, “Intravenous gammaglobulin (IVIG): a novel approach to improve transplant rates and outcomes in highly HLA-sensitized patients,” The American Journal of Transplantation, vol. 6, no. 3, pp. 459–466, 2006.

[14] A. D. Salama and C. D. Pusey, “Drug insight: rituximab in renal disease and transplantation,” Nature Clinical Practice Nephrology, vol. 2, no. 4, pp. 221–230, 2006.

[15] B. J. Nankivell, R. J. Borrows, C. L.-S. Fung, P. J. O’Connell, R. D. M. Allen, and J. R. Chapman, “The natural history of chronic allograft nephropathy,” The New England Journal of Medicine, vol. 349, no. 24, pp. 2326–2333, 2003.

[16] J. M. Halimi, M. Buchler, A. Al-Najar et al., “Urinary albumin excretion and the risk of graft loss and death in proteinuric and non-proteinuric renal transplant recipients,” The American Journal of Transplantation, vol. 7, no. 3, pp. 618–625, 2007.

[17] J. M. Halimi, “Low-grade proteinuria and microalbuminuria in renal transplantation,” Transplantation, vol. 96, no. 2, pp. 121–130, 2013.

[18] A. Djamali, M. Samaniego, J. Torrealba, J. Pirsch, and B. L. Muth, “Increase in proteinuria >200 mg/g after late rejection is associated with poor graft survival,” Nephrology Dialysis Transplantation, vol. 25, no. 4, pp. 1300–1306, 2010.

[19] N. Kieran, X. Wang, J. Perkins et al., “Combination of peritubular C4d and transplant glomerulopathy predicts late renal allograft failure,” Journal of the American Society of Nephrology, vol. 20, no. 10, pp. 2260–2268, 2009.

[20] B. Sis, P. M. Campbell, T. Mueller et al., “Transplant glomerulopathy, late antibody-mediated rejection and the ABCD tetrad in kidney allograft biopsies for cause,” The American Journal of Transplantation, vol. 7, no. 7, pp. 1743–1752, 2007.

[21] A. Vongwiwatanaa, S. Gourishankara, P. M. Campbella, K. Solez, and P. F. Hallorana, “Peritubular capillary changes and C4d deposits are associated with transplant glomerulopathy but not IgA nephropathy,” The American Journal of Transplantation, vol. 4, no. 1, pp. 124–129, 2004.

[22] R. N. Smith, F. Malik, N. Goes et al., “Partial therapeutic response to rituximab for the treatment of chronic alloantibody mediated rejection of kidney allografts,” Transplant Immunology, vol. 27, no. 2–3, pp. 107–113, 2012.

[23] C. A. Vieira, A. Agarwal, B. K. Book et al., “Rituximab for reduction of anti-HLA antibodies in patients awaiting renal transplantation: I. Safety, pharmacodynamics, and pharmacokinetics,” Transplantation, vol. 77, no. 4, pp. 542–548, 2004.