Impact of Subclinical Rejection on Kidney Graft Function

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ABSTRACT

Background. In kidney transplant recipients with borderline infiltration, protocol biopsy results demonstrated the relationship with chronic injury. The purpose of this study was to evaluate the effect of subclinical rejection (SCR) on 6-month protocol biopsy results in long-term renal function in renal transplant recipients with stable graft function.

Material and Methods. Transplant protocol biopsies performed in 45 patients with stable renal function were included in this study at 6 months. Biopsy specimens were evaluated for SCR. Study groups were divided into patients with and without SCR. Renal functions were compared with pathologic evaluation. The effect of immunosuppressive regimens on renal function were evaluated in patients with SCR

Result. The median age of patients was 32 years (range, 18-64 years). The median follow-up was 56 months (range, 24-84 months). According to the 6-month protocol biopsy results, 20 of 45 patients (44.4%) met SCR criteria based on Banff 07 parameters. There was not a statistically significant difference in renal function with SCR.

Conclusion. The presence of SCR on the 6-month protocol biopsy results in renal transplant recipients with a stable graft function does not cause deterioration in the long-term graft function.

IMMUNOLOGIC and nonimmunologic factors affect long-term graft function after kidney transplant [1]. Protocol biopsy results are important to reveal alloimmune response, drug toxicities, chronic organ damage, and viral effects. They have been routine follow-up protocols in some centers but not in many [1,2]. One of the possible factors for not using protocol biopsy in daily practice can be that sometimes clinical course and pathologic diagnosis does not correlate in patients with borderline findings. A current trend in recent years is treatment with pulse steroids, especially in the presence of a biopsy specimens with immune infiltration that are not correlated with the clinical findings [1–4]. Some studies demonstrated the relationship of this type of infiltration and fibrosis long-term [2,3,5–12]. In this respect, it is essential to detect presence of subclinical rejection (SCR) in those specimens.

Protocol biopsy results help us to reach much prognostic information on long-term graft function that cannot be determined by another method. The purpose of this study was to evaluate the effect of SCR of 6-month protocol biopsy results on long-term renal function in renal transplant recipients with stable graft function.

MATERIAL AND METHODS

This study was approved by the local ethical committee. Fifty-seven adult renal transplant recipients (transplant was performed between 2008 and 2012) with protocol biopsies at 6 months were evaluated

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retrospectively. Five patients who died with functional graft and 7 patients with a creatinine value above 2 mg/dL in first 6 months were excluded from the study. Finally, 45 renal transplant recipients with stable renal function were enrolled in the study.

The demographic data of patients and donors, cold ischemia time, presence of delayed graft function, number of HLA mismatches, and preoperative panel-reactive antibody levels were recorded. To assess the graft function, blood creatinine levels were recorded at time of discharge, time of biopsy, and years 1 to 5. In addition, patients' induction and maintenance immunosuppressive therapy and changes in immunosuppression protocols were also recorded.

Antithymocyte globulin (ATG) (Fresenius Biotech GmbH, Gräfelfing, Germany) or anti-interleukin 2 antibody (basiliximab [Simulect]) were used as induction therapy. Triple combinations such as calcineurin inhibitors (CNIs) (tacrolimus [Prograf], cyclosporine [Sandimmune, Neoral]), antiproliferative agents (mycophenolate mofetil [CellCept], mycophenolic acid [Myfortic]), and steroids or mammalian target of rapamycin (mTOR) inhibitors (sirolimus [Rapamune], everolimus [Certican]), antiproliferative agents, and steroids were preferred in maintenance of immunosuppression. Immunosuppression protocols were replaced with mTOR inhibitors in patients with adverse effects of CNI. Delayed graft function was considered to be need of hemodialysis in the first week after transplant.

Biopsy

Written consent was obtained from all patients before the procedure. All of the biopsies were performed by 18 G fully automatic biopsy needles under the guidance of ultrasonography. At least 2 cores were obtained. The patients were kept under observation for 24 hours after the biopsy.

Histologic Evaluation

Biopsy specimens were fixed by formaldehyde solution, and then 3-μm slices were prepared after rehydration and deparaffinization. Hematoxylin-eosin, Masson trichrome, periodic acid-Schiff, and silver staining were used for histologic examination. Specimens were evaluated by light microscopy. The slices with at least 5 glomeruli and 2 arterioles were evaluated. Biopsy samples were examined by 2 nephropathologists, and the decision was made together. Pathologic evaluation results were classified according to the criteria of Banff 07. Borderline changes that did not make the creatinine levels above 10% or grade IA scores were accepted as SCR [2,12,13]. Acute rejection (AR) was defined as AR-specific changes at protocol biopsy or clinical indication for biopsy in patients with graft dysfunction.

While specific treatment was not applied in patients with subclinical and borderline changes according to the biopsy results, ARs were treated by pulse steroids, and ATG was used in resistant cases. In the presence of antibody-mediated rejection, intravenous immunoglobulin and/or plasmapheresis was used.

Statistical Analysis

SPSS version 22 (IBM; Armonk, NY, United States) was used for whole statistical analysis. Data are reported as mean (standard deviation) and median (range), when appropriate. A P value less than .05 was considered significant. Fischer exact test and χ^2 test were performed to compare demographic covariates between groups, when appropriate. Mann-Whitney U test was used to assess differences in rank distributions of continuous variables between 2 groups.

Table 1. Comparison of Baseline Data and SCR

	SCR−	SCR+	*P* Value
Sex, No.			.13
Female	7	10	
Male	18	10	
Recipient age, mean (SD), y	34 (10) n = 25	35 (12) n = 20	.73
Living donor, No.	13	9	.64
Deceased donor, No.	12	11	
Donor age, mean (SD), y	34 (14) n = 25	40 (13) n = 20	.60
Marginal donor, No.	2	3	.45
CIT, median, min	550 n = 23	222 n = 17	.26
Follow-up time, mean (SD), mo	63 (15)	52 (11)	.29
Mismatches, mean (SD), No.	2.7 (1.4)	2.8 (1.7)	.31
Preoperative PRA, No.			.05
Positive	4	8	
Negative	15	7	
DGF (+)	8	6	.88
(−)	17	14	
Induction, No			.46
Simulect	14	9	
ATG	11	11	
Maintenance, No.			.69
CNI	23	19	
mTOR	2	1	
Maintenance, No.			.27
CNI TAC	16	10	
CsA	7	9	
Switch CNI/mTOR, No.	10	9	.74
Switch time, mean (SD), mo	8 (7) n = 10	7 (4) n = 9	.47
Acute rejection, No.	3	3	.77

Abbreviations: ATG, antithymocyte globulin; CIT, cold ischemia time; CNI, calcineurin inhibitor; CsA, cyclosporine; mTOR, mammalian target of rapamycin; PRA, panel-reactive antibody; SCR, subclinical rejection; TAC, tacrolimus.

RESULTS

The median age of patients was 32 years (range, 18-64 years). A total of 62.2% of patients were male (n = 28). The median follow-up time was 56 months (range, 24-84 months). Delayed graft function was observed in 14 patients (31.1%). ATG and interleukin 2 receptor antibody were applied to 22 and 23 patients as an induction therapy, respectively. CNI (tacrolimus: 26; cyclosporine: 16) and mTOR inhibitors (n = 3) were used as a maintenance immunosuppression therapy. AR was observed in 6 patients (13.3%) meanly at 2 months (range, 1-27 months). The median donor age was 40 years (range, 10-62 years), and median cold ischemia time was 647 minutes (range, 45-1320 minutes).

According to the 6-month protocol biopsy results, 20 of 45 patients (44.4%) met SCR criteria based on Banff 07 parameters. There was not a statistically significant difference between SCR+ and SCR− groups among demographics and baseline data ($P > .05$). Data of donor and transplant patients associated with SCR are summarized in Table 1.

Blood creatinine levels of patients at discharge time, month 6, and years 1 to 5 were compared to assess the

Table 2. Relation Between SCR and Serum Creatinine Levels

	SCR−	SCR+	*P* Value
Creatinine at discharge, mean (SD), mg/dL, mg/dL	1.34 (0.36) n = 25	1.33 (0.39) n = 20	.923
Creatinine at 6 mo, mean (SD), mg/dL	1.30 (0.3) n = 25	1.33 (0.29) n = 20	.44
Creatinine at 1 y, mean (SD), mg/dL	1.28 (0.41) n = 25	1.40 (0.51) n = 20	.40
Creatinine at 2 y, mean (SD), mg/dL	1.27 (0.4) n = 25	1.33 (0.46) n = 20	.53
Creatinine at 3 y, mean (SD), mg/dL	1.23 (035) n = 24	1.35 (0.41) n = 19	.60
Creatinine at 4 y, mean (SD), mg/dL	1.33 (0.42) n = 19	1.40 (0.46) n = 12	.64
Creatinine at 5 y, mean (SD), mg/dL	1.41 (0.40) n = 16	1.32 (0.53) n = 4	.72

Abbreviation: SCR, subclinical rejection.

relationship between graft function and SCR. Blood creatinine levels were minimally higher in the SCR+ group than in the SCR− group. However, this was not a statistically significant difference between the 2 groups (Table 2).

Immunosuppression protocols were switched to mTOR inhibitors from CNI in 19 patients during follow-up. Median switch time was 6 months (range, 1-24 months). Serum creatinine changes in patients with or without the switch is summarized in Table 3. Comparison of serum creatinine levels in patients with SCR according to CNI or mTOR inhibitors group are shown in Table 4.

Hemodialysis was needed for 1 patient who did not respond to treatment of AR at 24 months in follow-up. This patient had no signs of SCR in protocol biopsy results.

Table 3. Serum Creatinine Changes in Patients With or Without Switch

	Switch−	Switch+	*P* Value
Creatinine at discharge, mean (SD), mg/dL	1.26 (0.33) N = 24	1.38 (0.41) N = 18	.26
Creatinine at 6 mo, mean (SD), mg/dL	1.26 (0.32) N = 24	1.35 (0.29) N = 18	.30
Creatinine at 1 y, mean (SD), mg/dL	1.28 (0.47) N = 24	1.37 (0.48) N = 18	.45
Creatinine at 2 y, mean (SD), mg/dL	1.21 (0.41) N = 24	1.33 (0.40) N = 18	.12
Creatinine at 3 y, mean (SD), mg/dL	1.21 (0.37) N = 24	1.32 (0.37) N = 17	.15
Creatinine at 4 y, mean (SD), mg/dL	1.31 (0.47) N = 17	1.40 (0.38) N = 13	.30
Creatinine at 5 y, mean (SD), mg/dL	1.29 (0.46) N = 11	1.51 (0.37) N = 8	.13

Table 4. Comparison of Serum Creatinine Levels in Patients With SCR According to CNI or mTOR Group

	CNI Group	mTOR Group	*P* Value
Creatinine at discharge, mean (SD), mg/dL	1.22 (0.24) N = 10	1.42 (0.52) N = 9	.497
Creatinine at 6 mo, mean (SD), mg/dL	1.32 (0.28) N = 10	1.32 (0.33) N = 9	.84
Creatinine at 1 y, mean (SD), mg/dL	1.37 (0.46) N = 10	1.40 (0.61) N = 9	.97
Creatinine at 2 y, mean (SD), mg/dL	1.26 (0.40) N = 10	1.36 (0.54) N = 9	.497
Creatinine at 3 y, mean (SD), mg/dL	1.34 (0.35) N = 10	1.31 (0.49) N = 8	.90
Creatinine at 4 y, mean (SD), mg/dL	1.40 (0.44) N = 7	1.42 (0.53) N = 5	> .99
Creatinine at 5 y, mean (SD), mg/dL	1.06 (0.15) N = 3	2.1 (0) N = 1	.50

Abbreviations: CNI, calcineurin inhibitor; mTOR, mammalian target of rapamycin; SCR, subclinical rejection.

DISCUSSION

The most remarkable finding of the present study is that SCR findings obtained after 6-month protocol biopsy results in transplant recipients with stable kidney function did not cause adverse changes on kidney function long-term.

SCR is one of the most common and important histologic diagnoses that can be observed in protocol biopsy samples. SCR incidence is very high in the first months, and it is decreased at the end of the first year after transplant [2,12,14–17]. Renal dysfunction after SCR is observed usually after 6 to 12 months [18,19]. It has been accepted that SCR is related to progressive chronic graft damage, and when these 2 entities exist together, creatinine clearance and graft survival was affected adversely [10,15,16,17,20]. It is important to keep in mind that there can be difference in pathologists' assessments about SCR diagnosis [2]. Gough et al reported that interobserver reliability about SCR diagnosis was 42.9% among 2 independent pathologists who worked at 2 different transplantation centers [21,22].

SCR frequency at protocol biopsies in the first 6 months is reported as 17% to 60% in the literature [3-7,9,10,23]. Nankivell et al divided SCR into acute and borderline subgroups. The authors stated that borderline SCR rates were 7% to 44%, and acute SCR (Banff IA and above) rates were 3% to 50% at the different times of first year [2]. These findings confirm that there are significant differences between the centers about the SCR rates. SCR frequency in our study was determined to be 44.4%. Our opinion is that considering borderline changes and grade IA infiltrations together is the reason of our high SCR rates.

The general approach for the treatment of SCR at protocol biopsy is pulse steroid therapy [1–4,9,16]. Some studies suggest that SCR without treatment has no effect on kidney graft function in the midterm period; however, there are some opposite reports in the literature as well [2,6,7,9,12,15,24–26]. In a prospective study Rush et al reported that the treatment of SCR that was diagnosed in the

first 3 months reduced early and late rejection episodes and had an advantage on graft function at the 24th month [4]. Matoza et al indicated that partial response was observed in most of the patients with a diagnosis of borderline AR treated by pulse steroid, but there was no statistically significant difference in mean serum creatinine at 6 and 12 months whether patients received treatment [3]. Scholten et al suggested that the asymptomatic infiltrations that were observed on 6-month protocol biopsy specimens may not have harmful effect on the graft in midterm [7]. Beside these arguments, according to recent review, subclinical inflammations are independently associated with increased risk of chronic injury, despite treatment of SCR in the early period [14]. Significant effect on graft function in patients with SCR could not be confirmed in the present study.

It has been reported that treatment of the early SCR that is observed in first 3 months after transplant decreases the incidence of late clinic AR 3-fold [4]. Also, borderline infiltrations that were observed in the first 3 months' protocol biopsy specimens tended to be persistent, and AR can develop in one-third of the patients in the following months [27]. Borderline changes were observed in 81 of 351 patients (23%) with graft dysfunction at the biopsy with indication. It was reported that AR developed after progression in 55% of these patients with borderline changes who were not treated in the same study [27]. In contrast to all these findings, Gloor et al reported that borderline changes were observed in 13 of 114 patients (11%). Despite most of these patients having not received treatment, none of the patients had AR episodes in 6 and 27 months of follow-up [28]. Our study reveals that the incidence of AR is 13.3%, and the rejections were seen at a median 2 months after transplant. After comparing the groups with and without SCR, no difference in the development of AR was observed.

Superiority of tacrolimus to cyclosporine in preventing the cellular infiltration has been shown in many studies [3,10,12,20]. There is limited data about the SCR incidence in patients treated with sirolimus and everolimus. It has been reported that SCR incidence was quite high at the end of the first year, but the severity of the chronic lesions were decreased at the third year in patients treated with sirolimus [29]. The effects of CNI toxicity revealed in the longer term adversely affect the function of the kidney [30]. The use of mTOR inhibitors to avoid CNI toxicity has been promising in long-term graft function. However, there is some concern about the use of mTOR inhibitors, especially in patients with an AR history and with high immunologic risk [14,30]. A similar situation can also be considered in the patients with the SCR findings on protocol biopsy results who use mTOR inhibitors. In the present study, comparing the kidney functions between patients on CNI treatment and those who switched to mTOR inhibitors did not demonstrate a significant difference. Switching to mTOR inhibitors from CNI in patients with SCR has not shown a significant difference in kidney function.

In conclusion, the present study demonstrates that the presence of SCR on the 6-month protocol biopsy results in patients with stable graft function does not cause deterioration in the long-term graft function. This finding needs to be confirmed by further multicenter studies that are prospective, randomized, and include larger patient numbers.

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