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Original Article

Clinical Significance of Protocol Biopsy Soon after Renal Transplantation

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Background: Several studies reported that sub-clinical rejection (SCR) detected by a protocol biopsy soon after renal transplantation does permanent damage to a renal allograft, contributing to chronic allograft nephropathy (CAN). This article investigated the risk factors involved in SCR and the effects of treating SCR, and evaluated the clinical significance of a protocol biopsy soon after renal transplantation.

Methods: From January 2007 to June 2010, 253 patients received renal transplantation. Patients were divided into two groups according to whether or not they had undergone a protocol biopsy. To analyze the effect of SCR treatments, patients who were diagnosed with SCR were divided into two groups according to whether or not they had been treated with SCR. The patients who did not undertake a protocol biopsy were included in the untreated groups.

Results: Among 138 patients who undertook protocol biopsies, 65 patients (47.1%) showed SCR. In univariate analysis, both the number of HLA-DR mismatches (P=0.003) and not using Simulect (P=0.01) were identified as risk factors of SCR. In multivariate analysis, not using Simulect (P=0.006) was identified as an risk factor independent of SCR. δGFR, subtracting GFR at 1 week from GFR at that point, showed significant differences between SCR-treated patients and untreated patients at 1, 3, 6, 9, 12, 24, and 36 months with a P value of less than 0.05.

Conclusions: A protocol biopsy can detect SCR, especially in patients with risk factors such as a high number of HLA mismatches or not using Simulect. Treatment of SCR detected by protocol biopsy will help to improve long-term renal function.

Key Words: Protocol biopsy, Subclinical rejection (SCR), Renal transplantation
중심 단어: 프로토콜 생검, 준임상적 거부반응, 신이식

Introduction

Subclinical rejection (SCR) means histologically identified acute rejection with stable graft function(1). Previous studies have demonstrated that SCR found in protocol biopsy is common early after transplantation and do permanent damage to a renal allograft, contributing to chronic allograft nephropathy (CAN) (1-3). CAN is a leading cause of graft failure and is strongly correlated with the number of acute rejection episodes during the first year after renal transplantation(4). So the interests about the early detection and the treatment of SCR is increasing and this raises the possibility that protocol biopsies which are performed when graft function is stable may be clinically useful by allowing the detection of SCR. However, the risk of complications and unclear results of treatment in SCR patients make it difficult to perform protocol biopsy routinely.

The aim of this study was to evaluate clinical significance of protocol biopsy early after renal transplantation by identifying risk factors for SCR and the effect of anti-rejection therapy for SCR in renal transplantation.

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Materials and Methods

1) Patients

From January 2007 to June 2010, 337 renal transplantations were performed in Seoul National University Hospital. Among them, the patients who undertook a clinically-indicated biopsy because of acute rejection before 10 days after transplantation (n=23) and the patients under 18 years (n=61) were excluded. Remained patients were divided into two groups depending on whether protocol biopsies were performed or not; protocol biopsy (+) group (Protocol Bx (+)) (n=138) and protocol biopsy (−) group (Protocol Bx (−)) (n=115).

2) Immunosuppression

Immunosuppression protocol was a standardized triple therapy including one of calcineurin inhibitors (cyclosporine A, CsA or taclorimus, Tac), an antiproliferative agent (mycophenolatemofetil, MMF) and a prednisolon (PD). CsA was given targeting level of 100～250 ng/mL for the first 6 months and 40～80 ng/mL thereafter. Tac was given targeting 6～10 ng/mL for the first 6 months and 4～6 ng/mL thereafter. MMF was given 1,000～1,500 mg/kg per day, and PD was started at 1 mg/kg per day and was rapidly tapered to less than 5 mg per day(5). Before 2008, anti-IL2R monoclonal antibody (Simulect®; Novartis, Basel, Switzerland) was used in recipients who were performed deceased donor renal transplantation, had number of human leukocyte antigen (HLA) mismatches more than 3, and after 2008 it was used routinely.

3) Protocol biopsy

Ultrasono-guided needle biopsy was performed 10 days after transplantation. Protocol biopsies were performed using 18-gauge needle under ultrasound guidance. Three cores of tissue were obtained at each biopsy. Renal histopathological analysis was performed using the Banff97 classification(6), a semiquantitative scoring system used for the classification and grading of short and long term changes of a renal transplant. SCR was defined as histologically proven acute rejection without any graft dysfunction, as previously described. To assess feasibility of the protocol biopsy early after transplantation, complications (e.g., hemorrhage, peritonitis, graft loss) were evaluated.

4) Treatment of SCR

Anti-rejection therapy including methylprednisolone 0.5 g per day for 3 days was performed in the patients with evidence of acute T cell mediated rejection (TCR). In the patients with borderline change, some of them were treated and the others were not.

5) Statistics

Data was collected through retrospective review of medical records. Univariate analysis was carried out using the Student t test and the chi-square test. Multivariate analysis was carried out using the multiple logistic regression test. All P values were two sided, and a probability of less than 0.05 was considered significant.

Results

1) Demographics

During the study period, a total of 337 patients received kidney transplantation. Among them children under age 18 (n=61) and adult patients who diagnosed acute rejection before 10 days after transplantation (n=23) were excluded from this study. Demographic data are summarized in detail in Table 1. As a whole, patients undertook protocol biopsy were generally high risk patients. There were statistically significant differences in recipient age (45.15±13.30 vs 41.87±11.82, P=0.041), donor age (43.02±14.18 vs 37.62±10.40, P=0.001), type of donor (living donor : deceased donor, 43 : 94 vs 102 : 11, P<0.001), dialysis duration before renal transplantation (55.50±45.86 vs 27.78±34.34, P<0.001), and number of HLA mismatches (3.59±1.60 vs 3.05±1.50, P=0.007). As a result, induction with Simulect® was more frequent in patients with protocol biopsies.

Table 1. Demographics

	Protocol Bx (+) (n=138)	Protocol Bx (−) (n=115)	*P* value
Recipient sex, M : F (n)	75 : 63	65 : 50	0.413
Recipient age (yr)	45.15±13.30	41.87±11.82	0.041
Donor age (yr)	43.02±14.18	37.62±10.40	0.001
Dialysis duration	55.50±45.86	27.78±34.34	<0.001
Number of HLA mismatches (n)	3.59±1.60	3.05±1.50	0.007
HLA-A (n)	1.04±0.61	0.93±0.59	0.164
HLA-B (n)	1.36±0.72	1.16±0.67	0.021
HLA-DR (n)	1.20±0.655	0.97±0.66	0.004
Donor type, L : D (n)	43 : 94	102 : 11	<0.001
Tac use (n, %)	120 (87.0)	88 (76.5)	0.02
CsA use (n, %)	12 (8.7)	21 (18.3)	0.027
Simulect use (n, %)	108 (78.3)	70 (60.9)	0.011

Mean±SD.
Abbreviations: HLA, human leukocyte antigen; Tac, taclolimus; CsA, cyclosporine A.

Table 2. SCR prevalence in early protocol biopsy

		No. of patients	Prevalence (%)	Accumulative prevalence (%)
SCR	AMR	2	1.4	1.4
	Borderline change	46	33.3	34.8
	TCR - IA or IB	11	7.9	42.8
	TCR - IIA or IIB	6	4.3	47.1
NR		73	52.9	100
Total		138	100	100

Abbreviations: SCR, subclinical rejection; AMR, antibody mediated rejection; TCR, T-cell mediated rejection.

2) Prevalence of SCR

Protocol biopsies were performed 10 days after transplantation in 138 patients. Seventy three patients (52.9%) showed normal histology and 65 patients (47.1%) had subclinical rejection; acute antibody-mediated rejection in 2 patients (1.4%), borderline change in 46 patients (33.3%), and acute T-cell mediated rejection (TCR) in 17 patients (12.2%) (Table 2).

3) Risk factors of SCR

Sex and age of recipients, donor age, hypertension, diabetes mellitus, ABO mismatch, number of HLA mismatches, pre-transplant PRA, and immunosuppression protocol were enrolled for univariate analysis on risk factors of SCR. Among them, number of HLA-DR mismatches (*P*=0.127), 'not to use Simulect®' (*P*=0.01) were identified as risk factors for SCR (Table 3).

Table 3. Univariate analysis of risk factors for SCR

	NR (n=73)	SCR (n=65)	*P* value
Recipient sex, M : F (n)	36 : 37	39 : 26	0.208
Recipient age (yr)	44±13.29	45.52±13.26	0.915
Donor age (yr)	42.21±13.06	43.58±15.97	0.333
Dialysis duration	51.38±54.84	63.17±34.85	0.331
HTN (n, %)	13 (17.8)	9 (13.8)	0.526
DM (n, %)	3 (4.1)	5 (7.7)	0.369
ABO mismatch (n, %)	8 (11.0)	6 (9.2)	0.717
Number of HLA mismatches (n)	3.26±1.68	3.97±1.44	0.127
HLA-A (n)	1.03±0.67	1.05±0.54	0.101
HLA-B (n)	1.22±0.75	1.52±0.66	0.354
HLA-DR (n)	1.01±0.61	1.42±0.64	0.003
PRA>20% (n, %)	9 (12.3)	8 (12.3)	0.997
Donor type, L : D (n)	24 : 49	19 : 45	0.688
Immunosuppression			0.074
Tac/CsA+MMF+Pred (n, %)	67 (91.8)	64 (98.5)	
Others (n, %)	6 (8.2)	1 (1.5)	
Simulect use (n, %)	61 (83.6)	47 (72.3)	0.01

Abbreviations: NR, normal; SCR, subclinical reaction; HTN, hypertension; DM, diabetes mellitus; HLA, human leukocyte antigen; PRA, panel reactive antigen; Tac, tacloimus; CsA, cyclosporine A; MMF, mycophnolatemofetil; Pred, prednisolon.

Table 4. Multivariate analysis of risk factors for SCR: multiple logistic regression

	Odds ratio	95% CI	*P* value
Number of HLA mismatches (≤3 or >3)	1.195	0.292~4.890	0.805
HLA-A	0.498	0.220~1.128	0.095
HLA-B	2.062	0.851~4.995	0.109
HLA-DR	2.162	0.994~4.705	0.052
PRA>20%	1.445	0.437~4.778	0.546
Simulect use	0.219	0.074~0.650	0.006

Number of HLA mismatches and PRA already known as risk factors for SCR were included in analysis.
Abbreviations: HLA, human leukocyte antigen; PRA, panel reactive antigen.

Excluding the interactions of various factors, risk factors previously identified and number of HLA-A, B mismatches, PRA which had been known as risk factors of SCR already were enrolled for multivariate

analysis. 'Not to use Simulect®' (OR=0.219, 95% CI 0.074～0.650, *P*=0.006) was identified as an independent risk factor of SCR (Table 4).

4) Short and long-term renal function improvement

To prove the effect of treatment in SCR, patients were newly classified into two groups. Among the patients who undertook protocol biopsy, untreated patients despite of borderline change were divided into the same group with the patients who did not undertake protocol biopsy (Treatment (−) group, n=138). Other patients, who received treatment in the case of SCR as well as undertook protocol biopsy were divided into treated group (Treatment (+) group, n=43).

GFR at 1 week, 1, 3, 6, 9, 12, 24, and 36 months after transplantation were checked in all patients. GFR change (δGFR) before and after protocol biopsy were calculated by subtracting GFR at 1 week from GFR at that point. There were no significant differences between two groups in GFR (Fig. 1). However, there were significant differences between two groups in δ GFR at 1, 3, 6, 9, 12, 24 and 36 months with *P* value less than 0.05 (Fig. 2). The δGFR curve of treated group shows steady increase whereas δGFR curve of untreated group shows decrease after 12 months.

5) Complication of protocol biopsy

Among 138 patients underwent protocol biopsy, there were only 2 patients (1.4%) major complications in this study. One was bleeding required transfusion and the other was biopsy induced arteriovenous fistula in the renal parenchyme. There was no serious complication such as graft loss or patient death.

Discussion

Protocol biopsy has not been routinely performed because of its invasive character but some recent studies reported that protocol biopsy can provide benefits without major biopsy related complications(7-9). In this study, the complication rate was very low and only 2 patients (1.4%) suffered from biopsy-related complication.

According to previous studies, the incidence of SCR was reported to be 38% by Masin-Spasovska et al.(10) and 43% by Rush et al.(11). In this study, the incidence of SCR was 45.7%. Considering this high prevalence of SCR and its risk contributing to CAN, early detection of SCR with protocol biopsy and appropriate treatment should be required.

It is known that SCR is influenced by the time after transplantation, prior acute rejections, number of HLA mismatches, and immunosuppression(12). Previously known factors were not identified as independent risk factors of SCR in this study. This may be caused by the retrospective nature of this study. In addition, se-

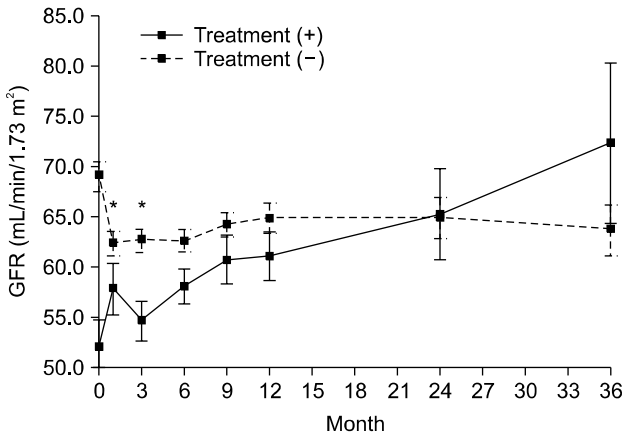

Fig. 1. Changes in mean MDRD GFR of each group. There were no significant differences between two groups in GFR after 3 months. **P*<0.05.

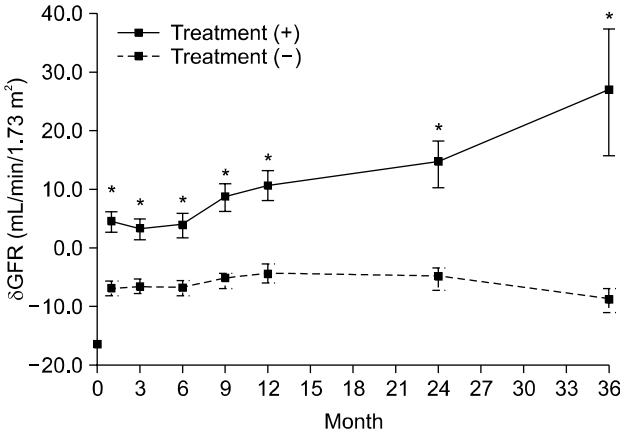

Fig. 2. δGFR defines as a result of subtraction MDRD GFR at 1 wk from MDRD GFR at the point. There were significant differences between two groups in δGFR at 1, 3, 6, 9, 12, 24 and 36 months. **P*<0.05.

lected patients with deceased donor or HLA mismatches greater than 2 were received Simulect® as an induction agent in the early period of this study.

According to previous studies demonstrating that steroid therapy for SCR patients can decrease of CAN(7), we did steroid pulse therapy for SCR patients. Need of management in patients diagnosed SCR such as additional anti-rejection treatment or immunosuppression change is still controversial(13-15). Rush et al.(11) reported that untreated SCR inflicts permanent tubulointerstitial damage and fibrosis. Nankivell et al.(1) reported that the treatment of SCR patients could reduce clinical acute rejection, one of the causes of CAN. Choi et al.(7) reported clinical significance of SCR in living donor kidney transplantation. They showed the group of patients with SCR in protocol biopsy had lower graft survival rate compared to the group of patients with normal or borderline change in protocol biopsy at 10% after 1～2 years, 20% after 5～6 years, 30% after 8～10 years. This study showed management of SCR can improve short and long-term renal allograft function. Therefore, detection and treatment of SCR is helpful for better renal transplant outcome.

After reports about the significance of treatment of SCR, researches on new method for diagnosis of SCR of renal allograft are continuing. Mao et al.(16) reported new non-invasive method, urine fingerprint analysis, but result of large-scale study will be needed for becoming a widely used test. Until the development of new methods to replace protocol biopsy, diagnosis and treatment of SCR through routine protocol biopsy must be considered to prevent CAN.

This study has several limitations. The most important limitation is that the two groups divided according to protocol biopsy were not identical. Because this study was retrospective analysis, two groups could not be assigned at random. As shown in Table 1, numbers of HLA mismatches of two groups known as a risk factor of SCR already were significantly different, and this factor could have been affected to the incidence of SCR in two groups. In order to compare renal function improvement of two groups, the adjustment of factors that may affect the incidence of SCR will be needed. Relatively small number of patients included in long term result analysis compared to number of patients included in short term result analysis also can be an another limitation.

Conclusion

In conclusion, protocol biopsy early after renal transplantation is safe enough to perform routinely. Due to the incidence of SCR is quite high, protocol biopsy should be considered more commonly in patients with risk factors of SCR such as high number of HLA mismatches, 'not to use Simulect®'. Treatment of SCR detected by protocol biopsy will help to make long-term renal function better.

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