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RESEARCH

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Effects of antirejection therapies for early subclinical acute rejection in renal transplant protocol biopsies

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Abstract

Background: Although recently strengthened immunosuppression protocols have decreased the incidence of clinical acute rejection of renal transplants, subclinical acute rejection and borderline changes remain problematic. This study was performed to evaluate the effects of antirejection therapies for early subclinical acute rejection and borderline changes.

Methods: In total, 269 renal transplant patients who received 3-month and 1-year protocol biopsies after renal transplantation were enrolled this study and divided into those with normal findings (Group A) and those with ≥ borderline changes (Group B) according to the 3-month pathological results. Pathological changes, graft function, and graft survival were evaluated at 1 year.

Results: The 3-month protocol biopsy revealed normal findings in 166 patients (Group A) and borderline changes and subclinical acute rejection in 103 patients (Group B). In Group A, 65.1% ($n=108$) of the patients maintained normal findings at 1 year, while 30.1% ($n=50$) deteriorated to ≥ borderline changes. In Group B, 52.4% ($n=54$) of patients improved to normal. Among patients with subclinical acute rejection, 25.0% ($n=5$) maintained subclinical acute rejection at 1 year despite antirejection therapy. The mean estimated glomerular filtration rate decreased from 60.4 ± 24.5 to 58.3 ± 19.0 mL/min/1.73 m^2 in Group A and from 57.2 ± 28.2 to 53.7 ± 20.3 mL/min/1.73 m^2 in Group B ($p=0.417$). The 3-, 5-, and 7-year graft survival rates were 99.4%, 99.4%, and 97.6% in Group A and 100.0%, 98.6%, and 98.6% in Group B, respectively ($p=0.709$).

Conclusions: Subclinical acute rejection is likely to recur. However, intervention for subclinical acute rejection in the early period after transplantation may help to prevent subsequent histological changes.

Keywords: Protocol biopsy, Renal transplantation, Subclinical acute rejection

Introduction

Recent advances in immunosuppression have reduced the rate of acute rejection (AR) after transplantation; however, AR remains problematic. Studies have shown that the rate of clinical AR has decreased to < 20% [1] and that biopsy-proven AR occurs in about 25% of patients [2, 3]. Subclinical AR (SAR) within 1 year post-transplantation has negative effects on graft outcomes, and untreated SAR is associated with development of chronic pathology [4].

A previous study demonstrated the efficacy of treatment for SAR in the early post-transplantation period [5]. However, "borderline changes" (BL) is an ambiguous diagnosis, and categorization of BL is difficult. Nankivell

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et al. [6] reported that BL without interstitial inflammation (i0) should not be managed as BL with interstitial inflammation (≥ i1) because the authors found no differences in the outcomes between a morphologically normal appearance and BL without interstitial inflammation. Therefore, it is difficult to treat BL as a type of AR [7], and treatment for BL remains unclear [6, 8–10].

We conducted paired protocol biopsies at 3 months and 1 year after renal transplantation. This study was performed to evaluate the morphological changes within 1 year post-transplantation and the efficacy of interventions based on the 3-month post-transplantation protocol biopsies.

Methods

Patient and clinical data

In total, 363 consecutive patients underwent renal transplantation from living related donors at our center from August 2006 to August 2016 (Fig. 1). All donors were adults (>20 years old), and their relationship with the recipient was a parent in 161 cases, spouse in 67 cases, sibling in 27 cases, child in 5 cases, grandparent in 3 cases, and third-degree relative in 6 cases. Paired protocol biopsies were performed at 3 months and 1 year post-transplantation in 332 of these 363 patients. We analyzed the association between the pathological results of the paired biopsies and antirejection therapy interventions. The exclusion criteria were clinical AR with antirejection therapy performed within 3 months after transplantation ($n=41$), existing preformed donor-specific antibody (DSA) before transplantation ($n=18$), and treatment with pretransplant plasmapheresis for focal segmental glomerulosclerosis ($n=4$).

Finally, 269 patients were enrolled. The patients were divided into two groups according to the histological changes at the 3-month protocol biopsy: patients with normal findings (NR) (Group A) and patients with ≥ BL changes (Group B). The patients with BL were subdivided into two groups: those who received antirejection therapy (BL-t, $n=33$) and those who underwent observation without therapy (BL-o, $n=50$). SAR was defined as protocol biopsy-proven AR (Fig. 1). We assessed the morphological changes between 3 months and 1 year post-transplantation and evaluated the efficacy of interventions for SAR and BL based on the 3-month post-transplantation protocol biopsy.

Immunosuppression therapy

For all patients, the maintenance immunosuppressants consisted of a calcineurin inhibitor (CNI) [cyclosporine (CYA) or tacrolimus (TAC)], an anti-proliferative agent (mycophenolate mofetil or azathioprine), and a tapering

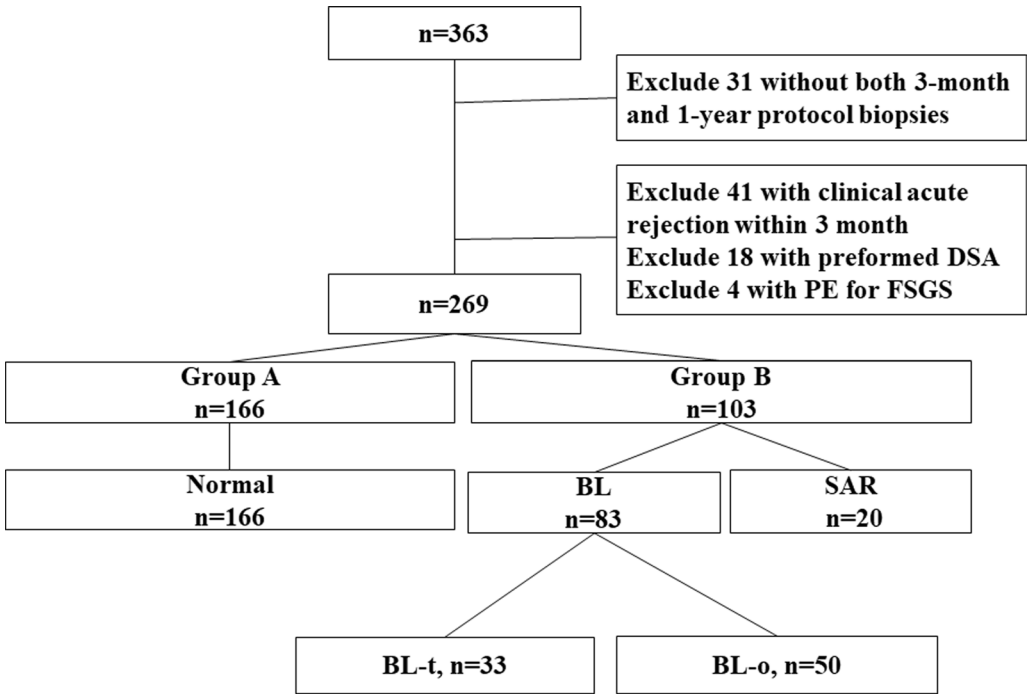

Fig. 1 Patient selection based on inclusion/exclusion criteria. DSA, donor-specific antibody; PE, plasma exchange; FSGS, focal segmental glomerulosclerosis; BL, borderline changes; SAR, subclinical acute rejection; BL-t, borderline changes with antirejection therapies; and BL-o, borderline changes with observation and no antirejection therapies

dose of methylprednisolone from 20 to 4 mg. The TAC dose was adjusted to maintain a trough value of 8 to 12 ng/mL until 1 month postoperatively and 7 to 10 ng/mL from 1 to 3 months. 3 months post-transplantation, the TAC dose was adjusted to maintain a trough of 5 to 7 ng/mL. The CYA dose was adjusted to achieve a measured area under the curve (AUC) of 0 to 4 h and target of 3500–4500 ng·h/mL until 1 month post-transplantation, AUC of 2500 to 3500 ng·h/mL until 3 months post-transplantation, and trough value of 100 to 150 ng/mL 3 months post-transplantation. Mycophenolate mofetil was administered at 25 to 30 mg/kg, but the AUC was not measured. Patients who underwent ABO-compatible transplantations received induction therapy with the anti-CD25 monoclonal antibody on day 0 and day 4 post-transplantation. For patients undergoing ABO-incompatible transplantations, plasmapheresis was performed one to three times according to the anti-A/B IgG titers, and anti-CD20 monoclonal antibody was administered at 10 days and 1 day preoperatively. The target titer was within 1:64 in adult recipients and 1:8 in pediatric recipients. When cytomegalovirus (CMV) infection was diagnosed, the reduction of immunosuppressive drugs was transient and was restored after recovery from the CMV infection.

Data collection

We retrospectively collected the following medical information: the demographic data of the recipient and donor, immunosuppressive agents, ischemic time, time of initial urine output, CMV infection, and graft survival. All patients were followed in our unit from transplantation to the last day of our observation. CMV infection was defined as the presence of CMV antigenemia. Prophylactic therapy for CMV infection was not available; only preemptive therapy was performed. All specimens were reviewed by at least two pathologists and one physician and were scored using recognized criteria according to the Banff classification [11]. Renal function was evaluated by the estimated glomerular filtration rate (eGFR). For pediatric patients (< 19 years of age), the eGFR was calculated using the fifth-order formula [12], and for adult patients (≥ 19 years of age), the eGFR was calculated using the Japanese Society of Nephrology formula (Eq. 194) [13]. The study protocol conformed to the Declaration of Helsinki and was approved by the ethics committee of Toho University Omori Medical Center (Approval Number M18207).

Treatment of SAR and BL

Intravenous methylprednisolone (250–500 mg/day for 3–5 days) and/or deoxyspergualin (3–5 mg/kg for 5–7 days) were administered as antirejection therapies. No patients in Group A received these antirejection therapies. In Group B, all patients with SAR received antirejection therapies. However, antirejection therapies for patients with BL were administered at the discretion of their attending physician considering their pathological severity and clinical course.

Statistical analysis

All values are presented as median [interquartile range (IQR)], mean and standard deviation, or percentage. Categorical variables were analyzed using the Chi-square test or Fisher's exact test. Continuous variables were compared using the unpaired t test, paired t test, and two-way analysis of variance. Median values were compared using the Wilcoxon rank sum test. Univariate and multivariate associations were analyzed using logistic regression analysis. Graft survival was assessed using the Kaplan–Meier method with the log-rank test. Differences were considered statistically significant at $p < 0.05$. The data were analyzed using JMP Pro software, version 13 (SAS Institute, Cary, NC, USA).

Results

Patients' backgrounds

The morphological changes at the 3-month post-transplantation protocol biopsies were studied in 269 patients (Group A, $n = 166$; Group B, $n = 103$) (Table 1). In Group B, BL and SAR were present in 83 and 20 patients, respectively. BL was divided into BL with antirejection therapies (BL-t, $n = 33$) and BL with observation and no antirejection therapies (BL-o, $n = 50$). AR also included grade 1a in 13 patients, grade1b in 2, grade 2a in 4, and grade 2b in 1. The number of patients receiving TAC was significantly higher in Group A than in Group B (54.2% vs. 39.8%, respectively; $p = 0.022$). The incidence of CMV infection within 3 months after transplantation was significantly higher in Group B than in Group A (50.5% vs. 28.3%, respectively; $p < 0.001$). A total of 61.7% and 66.7% of patients with CMV infection in Groups A and B, respectively, underwent reduction of their immunosuppression agents ($p = 0.608$). The pre-transplantation CMV serotype status (R−/D−, R−/D+, R+/D−, and R+/D+) was 7.9%, 21.2%, 4.2%, and 66.7% in Group A and 3.2%, 27.4%, 5.2%, and 64.2% in Group B, respectively ($p = 0.341$) (data not shown in table).

Pathological results of 3-month and 1-year post-transplantation protocol biopsies

The incidence rates of morphological changes are shown in Table 2. The rates at 3 months and 1 year post-transplantation were 61.7% and 60.2% in patients with NR, 30.9% and 32.7% in patients with BL, and 7.4% and 7.1% in patients with SAR. In Group A, 65.1% ($n = 108$) of

Table 1 Patient demographics and clinical characteristics

	Group A ($n=166$)	Group B ($n=103$)	*p*-value
Recipient sex, male	103 (62.5)	65 (63.1)	0.862
Recipient age, years	34 (11–63)	35 (13–48)	0.470
Donor sex, male	69 (41.6)	50 (48.5)	0.263
Donor age, years	50 (42–62)	53 (40–65)	0.723
HLA-A/B mismatch	1.69 ± 1.02	1.83 ± 1.07	0.278
HLA-DR mismatch	0.87 ± 0.64	0.96 ± 0.63	0.279
CNI (TAC)	90 (54.2)	41 (39.8)	0.022
ABO-incompatible	36 (21.7)	24 (23.3)	0.757
Plasmapheresis	17 (10.3)	18 (17.8)	0.078
Rituximab	36 (21.7)	22 (21.4)	0.949
WIT, min	3 (3–4)	4 (3–5)	0.100
CIT, min	66 (55–87)	65.5 (52–91)	0.333
Initial urine output, min	13 (7–28)	15 (9–29)	0.337
CMV infection within 3 months	47 (28.3)	52 (50.5)	< 0.001
IS decreased after CMV infection	61.7% (29/47)	66.7% (34/51)	0.608
3-month protocol biopsy			
Normal	166 (100)	0 (0.0)	
Borderline changes	0 (0.0)	83 (80.6)	
Subclinical acute rejection	0 (0.0)	20 (19.4)	

Data are presented as *n* (%), mean ± standard deviation, or median (interquartile range) unless otherwise indicated.

HLA human leukocyte antigen, *CNI* calcineurin inhibitor, *WIT* warm ischemia time, *CIT* cold ischemia time, *TAC* tacrolimus, *CMV* cytomegalovirus, *IS* immunosuppression agents

Table 2 Pathological results at 3 months and 1 year post-transplantation

3 months	1 year			*p*-value
	NR	BL	SAR	
Group A				
NR ($n=166$)	108 (65.1)	50 (30.1)	8 (4.8)	
Group B				
BL-o ($n=50$)	24 (48.0)	23 (46.0)	3 (6.0)	0.011
BL-t ($n=33$)	19 (57.6)	11 (33.3)	3 (9.1)	
SAR ($n=20$)	11 (55.0)	4 (20.0)	5 (25.0)	
Total ($n=269$)	162 (60.2)	88 (32.7)	19 (7.1)	

Data are presented as *n* (%).

NR normal findings, *BL* borderline changes, *SAR* subclinical acute rejection, *BL-t* borderline changes with antirejection therapies, *BL-o* borderline changes with observation and no antirejection therapies

patients still showed NR at 1 year post-transplantation, while 34.9% ($n=58$) had progressed to ≥ BL. In Group B, 41.0% ($n=34$) of patients with BL at 3 months post-transplantation still showed BL at 1 year post-transplantation, and 51.8% ($n=43$) had ameliorated to NR. Morphological improvement to NR occurred in 57.6% of patients in the BL-t subgroup but in only 48.0% of patients in the BL-o subgroup. The rate of NR at 1 year post-transplantation was lower in the BL-o subgroup than in the other groups. Among patients with SAR, 25.0% of patients still showed SAR at 1 year post-transplantation after receiving antirejection treatment. However, 55.0% ($n=11$) showed recovery to NR at 1 year post-transplantation. The CNI therapy was adjusted at 3 months according to the AUC or trough value based on our immunosuppressive protocols. Reduction of the CNI was performed for 121 (72.9%) patients in Group A and 67 (65.0%) patients in Group B (BL-o, $n=36$; BL-t, $n=23$; and SAR, $n=8$). However, the CNI was increased in 5 (3.0%) patients in Group A and 10 (9.7%) patients in Group B (BL-o, $n=2$; BL-t, $n=1$; and SAR, $n=7$).

Changes in tubulointerstitial inflammation score in patients with BL and SAR from 3 months to 1 year post-transplantation

Table 3 shows the histological changes in patients with BL and SAR according to the tubulitis (*t*) and interstitial inflammation (i) scores. There were significant differences across two time points for the three groups (*t* score, $p<0.001$; i score, $p=0.003$). At the 3-month post-transplantation protocol biopsies, the t score in the SAR group was significantly higher than that in the BL-o and BL-t subgroups (1.95 ± 0.76 vs. 1.16 ± 0.37 and 1.18 ± 0.39, respectively; all $p<0.001$). The t score

Table 3 Changes in tubulitis and interstitial inflammation scores in patients with borderline changes and subclinical acute rejection diagnosed at the 3-month protocol biopsy

	Score at 3 months	Score at 1 year
Tubulitis (*t* score)		
BL-o ($n=50$)	1.16 ± 0.37[a]	0.68 ± 0.71
BL-t ($n=33$)	1.18 ± 0.39[b]	0.45 ± 0.67
SAR ($n=20$)	1.95 ± 0.76[a,b]	0.60 ± 0.82
Interstitial inflammation (i score)		
BL-o ($n=50$)	0.68 ± 0.47[c]	0.53 ± 0.65
BL-t ($n=33$)	0.97 ± 0.47[d]	0.36 ± 0.55
SAR ($n=20$)	1.40 ± 0.82[c,d]	0.80 ± 0.83

Data are presented as mean ± standard deviation.

BL-o borderline changes with observation and no antirejection therapies, *BL-t* borderline changes with antirejection therapies, *SAR* subclinical acute rejection

[a] $p<0.001$

[b] $p<0.001$

[c] $p<0.001$

[d] $p=0.028$

in the BL-o, BL-t, and SAR groups was significantly lower at 1 year post-transplantation than at 3 months ($p<0.001$). With respect to interstitial inflammation, the i score in the SAR group was significantly higher than that in the BL-o and BL-t subgroups at 3 months post-transplantation (1.40 ± 0.82 vs. 0.68 ± 0.47 and 0.97 ± 0.47, respectively; $p<0.001$ and $p=0.028$). The BL-o and BL-t subgroups showed a significant reduction in the i score at 1 year post-transplantation ($p<0.001$). The pathological severity at 3 months post-transplantation and the pathological improvement at 1 year post-transplantation are shown in Table 4. In the BL group ($n=83$), these pathological findings at 1 year post-transplantation showed no differences between t1 and t2–3 or between i0 and i1 (Table 4).

Table 4 Prevalence of treatment for BL at 3 months and prevalence of histology showing NR and ≥BL at 1 year in the BL subgroup divided by severity of tubulitis and interstitial inflammation

	Treatment at 3 months	1 year		*p*
		NR	≥BL	
Tubulitis (*t* score) at 3 months				
*t*1 ($n=69$)	27 (39.1)	34 (49.3)	35 (50.7)	0.385
t≥2 ($n=14$)	6 (42.9)	9 (64.3)	5 (35.7)	
Interstitial inflammation (i score) at 3 months				
*i*0 ($n=20$)	4 (20.0)	11 (55.0)	9 (45.0)	0.801
i≥1 ($n=63$)	29 (46.0)	32 (50.8)	31 (49.2)	

Data are presented as n (%)

NR normal findings, *BL* borderline changes

In the comparison of the BL-o and BL-t subgroups, there were no differences in the demographic data of the recipient and donor, immunosuppressive agents, cold ischemia time, time of initial urine output, or CMV infection within 3 months. However, donor–recipient human leukocyte antigen mismatch (HLA-DR mismatch) (BL-o, 0.84 ± 0.62; BL-t, 1.12 ± 0.60; $p=0.043$) and the warm ischemia time [BL-o, 4 min (IQR, 3–5); BL-t, 3 min (IQR, 3–4); $p=0.012$] showed statistically significant differences. Univariate and multivariate associations with the pathological change to NR in the BL group were analyzed. Our univariate analyses showed that the pathological change to NR was not influenced by the therapeutic intervention for BL [odds ratio (OR), 1.47; 95% confidence interval (CI), 0.61–3.56; $p=0.394$], HLA-DR mismatch (OR, 1.47; 95% CI, 0.72–3.01; $p=0.279$), or the warm ischemia time (OR, 1.05; 95% CI, 0.77–1.41; $p=0.772$). In the multivariate analysis, intervention for BL (OR, 1.26; 95% CI, 0.48–3.36; $p=0.623$), HLA-DR mismatch (OR, 1.51; 95% CI, 0.72–3.21; $p=0.274$), and the warm ischemia time (OR, 1.02; 95% CI, 0.77–1.36; $p=0.870$) were also not associated with the pathological change to NR.

Graft function and graft survival rate

The eGFR decreased from 60.4 ± 24.5 to 58.3 ± 19.0 mL/min/1.73 m^2 ($p=0.042$) in Group A and from 57.2 ± 28.2 to 53.7 ± 20.3 mL/min/1.73 m^2 ($p<0.01$) in Group B (Fig. 2). However, the eGFR did not show sequential differences between the two groups ($p=0.417$). The eGFR was numerically lower in the BL-o than BL-t subgroup at 3 months and 1 year post-transplantation (55.9 ± 21.1 vs. 62.0 ± 40.6 mL/min/1.73 m^2 and 53.2 ± 18.1 vs. 56.9 ± 26.6 mL/min/1.73 m^2, respectively; $p=0.438$). The 3-, 5-, and 7-year graft survival rates were 99.4%, 99.4%,

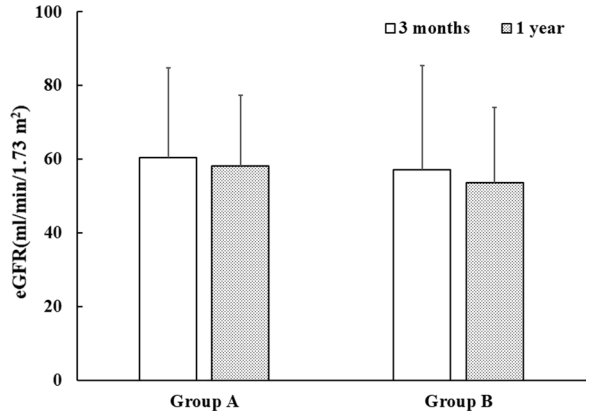

Fig. 2 Changes in mean eGFR. eGFR, estimated glomerular filtration rate

and 97.6% in Group A and 100.0%, 98.6%, and 98.6% in Group B, respectively ($p=0.709$) (Table 5). Graft loss occurred in three patients (one in Group A and two in Group B). The cause of graft loss was chronic allograft nephropathy in all patients. In Group B, these graft survival rates were 100%, 100%, and 100% in the BL-t subgroup; 100%, 97.1%, and 97.1% in the BL-o subgroup; and 100%, 100%, and 100% in the SAR subgroup.

Discussion

This study revealed the significance of early intervention based on the pathological results of an early protocol biopsy. Overall incidence rates of BL and SAR were similar at 3 months and 1 year post-transplantation. Moreover, subsequent pathological changes were more likely to be the same as the preceding pathological changes. Antirejection therapies showed an advantage with respect to amelioration for subsequent histological changes.

Consistent with our results, Kee et al. [2] reported that AR and BL were detected in 12.5% and 34.1% of their protocol biopsies ($n=88$). Early SAR may occur for several reasons, including HLA mismatch, the type of immunosuppression agents used, under-immunosuppression, infection, delayed graft function, and donor age [14–16]. Choi et al. [16] reported that the incidence of SAR increased as the number of HLA-DR mismatches increased in living-donor renal transplantation. They studied 304 patients who underwent transplantation and found that the number of HLA-DR mismatches was significantly higher in patients with SAR detected in the 2-week post-transplantation biopsies (OR, 2.39) [16]. In our study, the numbers of HLA-A/B and HLA-DR mismatches were more likely to be higher in patients with ≥ BL than in those with NR at 3 months post-transplantation. The selection of immunosuppression agents is also important to prevent SAR. Our study also showed that TAC treatment was associated with a significantly higher incidence of histologically NR than ≥ BL at 3 months post-transplantation. Mateu et al. [17] studied 3365 kidney transplant recipients and found that treatment with CYA increased the risk of AR compared with TAC (risk ratio, 1.57; $p=0.007$) [17].

Table 5 Graft survival rate

3 years	5 years	7 years	*p*-value
Group A			
146 (99.4)	92 (99.4)	48 (97.6)	0.709
Group B			
93 (100.0)	69 (98.6)	58 (98.6)	

Data are presented as *n* (%)

AR is known to be an indirect effect of CMV [18]. Previous studies have shown that CMV infection within 1 month after transplantation is a risk factor for AR (risk ratio, 1.6; $p=0.020$) [19]. Another report focused on the relationships between SAR and CMV disease or CMV infection in 106 recipients who were seropositive for CMV before transplantation [20]. CMV disease was a risk factor for AR (hazard ratio, 3.0; $p=0.014$), but asymptomatic CMV infection was not ($p=0.987$). Conversely, our study showed that recipients with BL and SAR at 3 months post-transplantation were more likely to exhibit positive CMV antigenemia within 3 months after transplantation. Although there were no significant differences in the reduction rate of immunosuppression drugs after CMV infection between the SAR/BL group and the normal group (61.7% vs. 66.7%, $p=0.608$), we speculate that the reduction of immunosuppression following CMV infection might affect the occurrence of SAR or BL at the 3-month protocol biopsy. To resolve early SAR and BL associated with CMV infection, prophylactic therapy for CMV infection may act to prevent CMV infection and reduce early SAR and BL.

The incidence of SAR within 1 year post-transplantation is associated with graft survival [3]. Zachariah et al. [21] found that SAR and BL diagnosed by late biopsy (12–24 months) were significantly associated with a decreased eGFR. Choi et al. [16] reported that the 10-year graft survival rates in patients with NR, BL, and untreated SAR were 96.2%, 93.2%, and 62.3%, respectively. Protocol biopsies have an advantage in detecting unrecognized morphological changes in grafts even when no symptoms are present. Kurtkoti et al. [5] conducted a prospective randomized study to compare patients who did and did not receive 1- and 3-month post-transplantation protocol biopsies, and the authors found that the patients who received the protocol biopsies showed a lower serum creatinine concentration at 6 months (1.28 ± 0.33 vs. 1.55 ± 0.39 mg/dL, $p<0.001$) and 12 months (1.20 ± 0.33 vs. 1.52 ± 0.41 mg/dL, $p<0.001$). Early detection of post-transplantation morphological changes using protocol biopsies appears to be important, and it contributes to early treatment and prevention of BL or SAR to avoid impairment of graft outcomes.

The treatment efficacy for protocol biopsy-proven SAR has been revealed in previous reports [4, 22]. Miyagi et al. [22] found that high-dose steroid pulse treatment for early SAR was effective in suppressing the development of interstitial fibrosis at 1 year after transplantation. Although SAR affected the chronic score, it did not reflect the serum creatinine level in the short term after transplantation [22]. Rush et al. [4] demonstrated that untreated SAR and BL were associated with the development of early chronic pathology and late graft

dysfunction compared with treated SAR and BL. The serum creatinine level 2 years after transplantation was significantly lower in the treated SAR + BL group than in the untreated group (133 ± 14 vs. 188 ± 22 μmol/L, respectively; $p = 0.05$). Consequently, the authors suggested that antirejection treatment for SAR including BL should be administered to improve the long-term pathological and graft prognosis [4].

Treatment for BL remains controversial. A consensus regarding the optimal treatment for BL has not been established [8] because BL is an ambiguous diagnosis and is difficult to treat as a type of rejection [7]. McRae et al. [9] reported that tubulitis with interstitial inflammation (BLt1i0) detected in the indication biopsy had a prognosis similar to that of normal histological change, bringing into question the current diagnostic thresholds for BL. In the category of BL, tubulitis with interstitial inflammation (BLt1i1) and without interstitial inflammation (BLt1i0) shows a different prognosis; BLt1i1 is more likely to be associated with deteriorated graft function and graft failure than is BLt1i0. This result indicates that BLt1i1 is histologically similar to AR. In the present study, patients with BL who developed severe interstitial inflammation were more likely to receive antirejection therapies. In the BL-o group, both the i score and t score were decreased at 1 year post-transplantation, but both scores were higher than those in the BL-t group. The antirejection therapies in the BL-t group seemed to be effective; however, whether these therapies should be administered in the BL-o group remains unclear, and further study is needed.

The main limitations of this study are the short-term observation period and the small number of patients from a single center. As mentioned above, the treatment for BL was not randomized. Among patients with BL at 3 months, those who received treatment had more interstitial inflammation than those who did not receive treatment. Additionally, there was no predefined protocol of antirejection therapies, including methylprednisolone and/or deoxyspergualin. Consequently, the treatment was performed according to the subcategorized grade of BL and each patient's clinical condition. DSA was not analyzed in this study because we could not collect DSA in all cases. Thus, any associations between early histological changes and DSA could not be determined because of the lack of DSA results. Notably, graft survival was high and graft function was basically stable throughout the 1-year period, and no clinical AR-related events occurred. Thus, comparison of the graft prognoses showed only minor differences.

In conclusion, detection of early histological changes by protocol biopsy and the performance of interventions based on these changes may contribute to improvement of subsequent histological changes. However, SAR (including BL) is more likely to recur, and careful observation is needed for patients with these histological changes to ameliorate the graft prognosis.

Abbreviations

AR: Acute rejection; AUC: Area under the curve; BL: Borderline changes; CI: Confidence interval; CMV: Cytomegalovirus; CNI: Calcineurin inhibitor; CYA : Cyclosporine; DSA: Donor-specific antibody; eGFR: Estimated glomerular filtration rate; HLA-DR mismatch: Donor–recipient human leukocyte antigen mismatch; IQR: Interquartile range; OR: Odds ratio; SAR: Subclinical acute rejection; TAC: Tacrolimus.

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Author contributions

KS wrote the manuscript. MM was a co-supervisor and designed and edited the manuscript. HO, NT, and TM contributed to the sample preparation and were involved in the pathological diagnosis. YI, TK, YH, SS, and KS discussed the results and contributed to the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data used in this article are available from the corresponding author.

Declarations

Ethics approval and consent to participate

The study protocol adhered to the statutes of the Declaration of Helsinki and was approved by the ethics committee of Toho University Omori Medical Center (approval number M18207).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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