ORIGINAL ARTICLE

Two-year protocol biopsy after kidney transplantation in clinically stable recipients – a retrospective study

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SUMMARY

The idea of protocol biopsy is to detect subclinical pathologies, including rejection, recurrent disease, or infection for early intervention and adjustment of immunosuppressants. Nevertheless, it is not adopted by most clinicians because of its low yield rate and uncertain long-term benefits. This retrospective study evaluated the impact of protocol biopsy on renal function and allograft survival. A two-year protocol biopsy was proposed for 190 stable patients; 68 of them accepted [protocol biopsy (PB) group], while 122 did not [nonprotocol biopsy (NPB) group]. The rejection diagnosis was made in 13 patients by protocol biopsy, and 11 of them had borderline rejection. In the following 5 years, graft survival was better in the PB group than in the NPB group ($P = 0.0143$). A total of 4 and 17 patients in the PB and NPB groups, respectively, had rejection events proven by indication biopsy. Renal function was better preserved in the PB group than in the NPB group ($P = 0.0107$) for patients with rejection events. Nevertheless, the survival benefit disappeared by a longer follow-up period (12-year, $P = 0.2886$). In conclusion, 2-year protocol biopsy detects subclinical pathological changes in rejection and preserves renal function by early intervention so as to prolong graft survival within 5 years.

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Key words
borderline rejection, kidney transplantation, protocol biopsy, subclinical rejection

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Introduction

Long-term survival for kidney transplant has not improved tremendously despite the progress in immunosuppressants [1]. Antibody-mediated rejection (ABMR) and glomerulonephritis (GN) remain the main causes of long-term graft failure [2]. The efficacy of ABMR treatment is unsatisfactory, especially with late allograft changes [3]. Protocol biopsy provides an opportunity to identify early pathological changes in rejection for early intervention. Nevertheless, in the post-Symphony study era, patients have a lower rejection rate under the standard regimen of tacrolimus, mycophenolate mofetil, and steroids [4,5]. Early protocol biopsy within 6 months after transplantation has been reported to be associated with a low prevalence of subclinical rejection but to have no benefit in prognosis [6–8]. Thus, in a surveillance of transplant centers across the USA, <20% performed protocol biopsy because of a low yield rate and uncertain advantage [9]. A recent report on a subsequent late protocol biopsy between 1 and 2 years, however, revealed that the

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biopsy results could predict long-term allograft function [10].

In the National Taiwan University Hospital (NTUH), we proposed the 2-year protocol biopsy to all clinically stable patients, some of whom agreed to undergo the procedure. Both subclinical and borderline rejections based on protocol biopsy were treated. This retrospective study aimed to examine whether a late (2-year) protocol biopsy policy could improve long-term renal function and graft survival.

Patients and methods

Patients

We reviewed the data of adult patients who underwent kidney transplantation between January 2007 and December 2013 (Fig. 1). We first excluded sensitized patients who were ABO-incompatible or with preformed donor-specific antibodies. These patients have a higher risk of rejection, and most underwent indication biopsy within 2 years after transplantation (Fig. S1). Additionally, patients who underwent indication biopsy within 2 years (Fig. S2) after transplantation and those with malignancy (Table S1) were also excluded. Early indication biopsy resulted in patients receiving variable treatment courses according to the biopsy results, and patients with malignancy received different systemic therapies. Finally, a cohort of clinically "stable" patients 2 years after transplantation was generated. These patients were divided into two groups: the protocol biopsy (PB; patients who underwent 2-year protocol biopsy) and the nonprotocol biopsy (NPB) group.

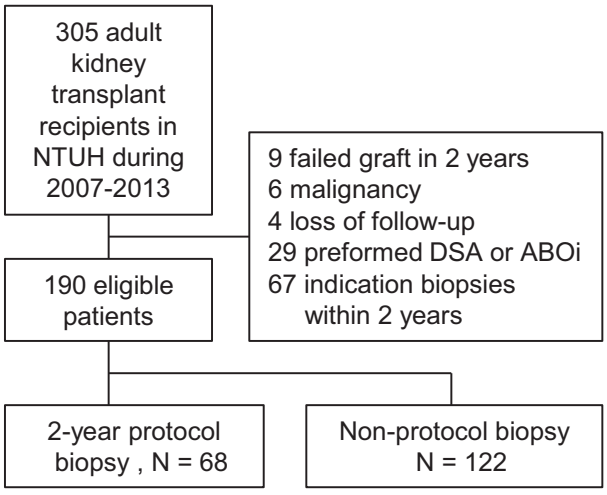

Figure 1 Flowchart of patient selection in the study cohort. ABOi, ABO-incompatible transplantation; DSA, donor-specific antibody

Indication biopsies were performed if creatinine elevation (>20%), or significant proteinuria (24-h urine total protein >0.5 g) was observed in the patients. Results of the indication biopsy within 7 years after transplantation were reviewed for analysis. This retrospective study was approved by the Research Ethics Committee of the NTUH (201910089RINB).

Data collection

Information on the patients' basic characteristics and parameters of kidney transplantation, including date, donor profiles, matching results, renal function, and immunosuppressant usage, was obtained. Renal function was presented by the estimated glomerular filtration rate (eGFR), which was calculated using the four-variable modification of diet in renal disease formula. To monitor medication compliance, the trough level of immunosuppressants (tacrolimus, cyclosporine, or sirolimus) in the second year was identified to calculate the standard deviation (SD). The percent coefficient (CV%) was calculated as follows: SD/mean × 100%. Kidney transplant loss was defined as a return to dialysis.

Maintenance of immunosuppressants and treatment for rejection

After transplantation, the tacrolimus level was initially maintained at 5–8 ng/ml, with mycophenolate mofetil 500 mg twice a day and prednisolone 5 mg/day. If side effects were noted, we adjusted the dose or regimen according to the patient's response.

After protocol or indication biopsy, both borderline rejection (BL-R) and definite T-cell-mediated rejection (TCMR) were treated with pulse steroid therapy with methylprednisolone 500 mg/day in three doses. For ABMR, treatment includes four sessions of double-filtration plasmapheresis (DFPP) and intravenous immunoglobulin, which was administered immediately after every DFPP at a dose of 0.5 g per day [11].

Pathology

All pathological reports were reviewed by a single pathologist (WC Lin). We developed the pathological scoring and made the diagnosis for both protocol and indication biopsy specimens using the contemporary Banff 2017 classification guideline.

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Statistical analysis

All numbers are presented as mean ± SD. An unpaired *t*-test with Welch's correction was used to compare nominal data sets. Data proportions were compared using Fisher's exact test or chi-squared test. For both death-censored and rejection-free graft survivals, we presented the data using the Kaplan–Meier curves and analyzed them using the log-rank test. Risk factors for graft survival were analyzed with a stepwise regression model. Two-sided P values < 0.05 were considered statistically significant. Statistical analysis was performed using the software GraphPad Prism 8.4.2 (GraphPad Software, LLC, San Diego, CA, USA) or Stata statistical software, Release 14, (StataCorp LLC, College Station, TX, USA).

Results

Demographic data of the patients

This study included 190 out of 305 patients who underwent kidney transplantation between 2007 and 2013 (Fig. 1). There were nine allograft failures within 2 years, and four patients were lost to follow-up. Six patients had malignancies diagnosed after transplantation, and 4 (66.67%) of these patients died with functioning grafts within 5 years (Table S1). To verify the clinical effects of protocol biopsy, we also excluded two groups of patients to create a more homogenous study cohort. Patients with preformed donor-specific antibody or ABO blood type incompatibility ($n = 29$) were excluded because they have a higher risk of rejection events and most had indication biopsy within 2 years with variable treatment courses (Fig. S1). Patients who underwent indication biopsy within 2 years ($n = 67$) were excluded because these patients had variable confounding factors following different therapies according to their biopsy results (Fig. S2). Therefore, the patients included in the study cohort had stable renal function with nonsignificant proteinuria 2 years after transplantation. A protocol biopsy was proposed at the end of the second year, and 68 patients (35.79%) agreed to this procedure. Between the patients who underwent protocol biopsy (PB group) and those who did not (NPB group), no difference in sex ratio, primary disease, immunological profiles before transplantation, and immunosuppressant regimens was noted (Table 1). Patients were younger (38.76 ± 13.25 vs. 45.54 ± 11.74; $P = 0.0007$), and there were more recipients of living donor kidney transplant (69.12% vs. 33.61%; $P < 0.0001$) in the PB group than in the NPB group. It is known that medication noncompliance is associated with rejection, and variation in blood immunosuppressant levels (calculated as CV%) was reported to be a good marker [12]. In the second year, no difference in CV% was found between the two groups (24.38 ± 13.14 vs. 22.61 ± 13.86, PB vs. NPB; $P = 0.3863$).

Graft survival and renal function 5 years after protocol biopsy

In the PB group, 13 patients had pathological findings of rejection (Fig. 2), including BL-R, TCMR, and ABMR. All patients were treated according to the pathological results. In the following 5 years (i.e., third to seventh year after transplantation), three patients who had BL-R by PB had ABMR by indication biopsy. In addition, among patients with negative PB results, only one was diagnosed as having BL-R by indication biopsy.

Rejection diagnosis by indication biopsy during the third to seventh year was higher in the NPB group (17/122, 13.93%) than in the PB group (4/68, 5.88%; Fig. 2); however, the difference was not statistically significant ($P = 0.0981$). Although the overall rejection events were higher in the PB group (Fig. 3a), the death-censored 7-year graft survival was higher in the PB group than in the NPB group (100% vs. 91.49%; $P = 0.0143$, Fig. 3b). There were more allografts from living donors in the PB group than in the NPB group (69.12% vs. 33.61%), which might be a confounding factor for survival. We then compared only the subgroup of living donor recipients. The result was similar: Death-censored 7-year graft survival was still higher in the PB group (100% vs. 84.95%, $P = 0.0030$, Fig. S3a). Moreover, multivariate regression analysis revealed that 7-year graft survival could be predicted by protocol biopsy, drug compliance (CV%), and better initial renal function (Table 2).

In our hospital, all patients with biopsy-proven rejections or BL-R were treated. The renal functions of the patients (gray background squares in Fig. 2) were compared (Fig. 3c). From the fourth year (2 years after protocol biopsy), renal function became significantly superior in the PB group and persisted until the seventh year of follow-up (mean eGFR, 51.93 ± 20.63 vs. 25.40 ± 19.00 ml/min/1.73 m^2; $P = 0.0107$), which corresponds to the survival curve.

Rejection and histologic characteristics

Among those with positive rejection findings in the protocol and indication biopsy groups, only one ABMR (7.69%) in the protocol biopsy group and 12 (57.14%) in

Table 1. Comparison of baseline characteristics between protocol biopsy and nonbiopsy groups

	Protocol biopsy (n = 68)	No protocol biopsy (n = 122)	P value
Donor			
Age (years)	43.94 ± 11.89	40.68 ± 11.83	0.0733
Male, n (%)	32 (48.48)	70 (57.38)	0.2838
Deceased donor (%)	21 (30.88)	81 (66.39)	<0.0001
eGFR (ml/min/1.73 m^2)	91.92 ± 46.07	90.72 ± 37.50	0.8575
Recipient			
Age (years)	38.76 ± 13.25	45.54 ± 11.74	0.0007
Male/female, n	35/33	68/54	0.6491
Cause of ESRD, n (%)			
GN	19 (27.94)	28 (22.95)	0.8808
HTN	1 (1.47)	3 (2.46)	
Diabetes	2 (2.94)	3 (2.46)	
Other	11 (16.18)	17 (13.93)	
Unknown	35 (51.47)	71 (58.20)	
HLA mismatches, n	2.22 ± 1.27	2.23 ± 1.48	0.9650
Delayed function, n (%)	1 (1.47)	4 (3.28)	0.6563
Positive pre-tx PRA, n (%)			
Class I	11 (16.18)	10 (8.20)	0.1457
Class II	5 (7.35)	8 (6.56)	>0.9999
Maintenance IS, n (%)			
CNI + MMF	65 (95.59)	108 (88.52)	0.1186
mTOR + MMF	3 (4.41)	14 (11.48)	
Variation in drug level (CV%)	24.38 ± 13.14	22.61 ± 13.86	0.3863

CNI, calcineurin inhibitor; CV%, percent coefficient; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; GN, glomerulonephritis; HLA, human leukocyte antigen; HTN, hypertension; IS, immunosuppressant; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; PRA, panel-reactive antibody.

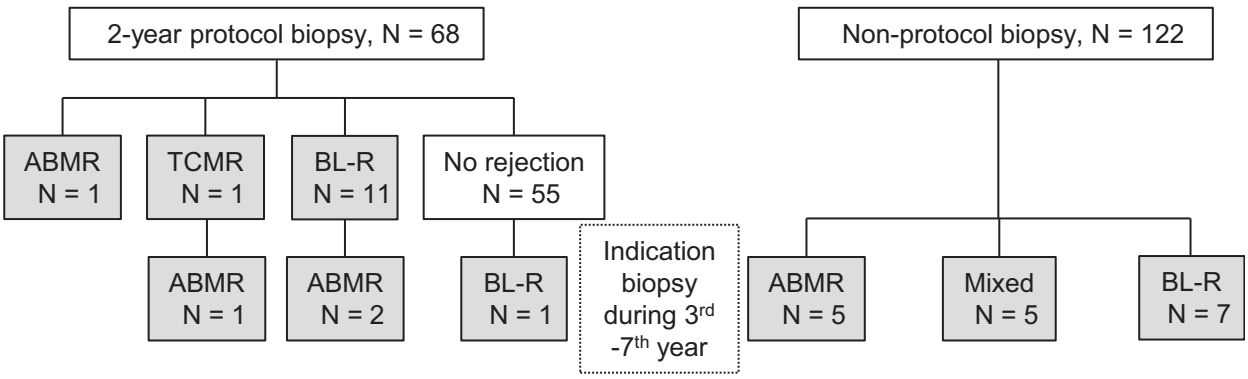

Figure 2 Biopsy results with rejection findings in the two study groups. ABMR, antibody-mediated rejection; BL-R, borderline rejection; TCMR, T-cell-mediated rejection

the indication biopsy group were noted. This finding reflects the Banff score, wherein patients undergoing indication biopsy had a greater score in complement deposition (C4d) and microvascular lesions (glomerulitis and peritubular capillaritis). Furthermore, the change in interstitial inflammation tended to be more severe in the indication biopsy group, although the difference was not statistically significant (P = 0.0599 for total inflammation and P = 0.0570 for inflammation). Tubulitis was not significantly different between the groups (Fig. 4). Details of the Banff scores are presented in Table S2.

Other histopathological findings of renal transplant

In addition to rejection, other etiologies that are significant for graft survival and renal functions were found by

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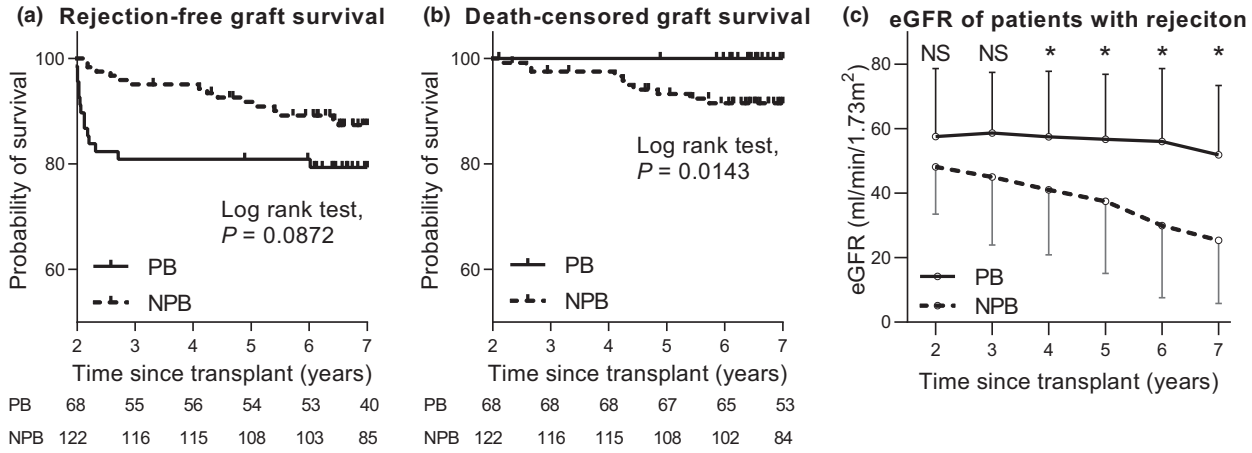

Figure 3 Comparison of survival and graft function. Kaplan–Meier curves were plotted for rejection-free (a) and death-censored (b) graft survivals. (c) Renal function evolution of patients with biopsy-proven rejection was compared after protocol biopsy. eGFR, estimated glomerular filtration rate; NPB, nonprotocol biopsy group; NS, not significant; PB, protocol biopsy group. *$P < 0.05$

Table 2. Clinical risk factors for 7-year graft failure by univariate and multivariate regression analyses

	Univariate			Multivariate		
	Coefficient	95%CI	*P* value	Coefficient	95%CI	*P* value
Recipient						
Sex (male vs. female)	0.0335	−0.0308 to 0.0978	0.306			
Age	−0.0001	−0.0026 to 0.0024	0.950			
Donor						
Sex (male vs. female)	0.0134	−0.0510 to 0.0778	0.683			
Age	0.0015	−0.0115 to 0.0042	0.261			
eGFR	−0.0003	−0.0011 to 0.0005	0.472			
Type (living vs. deceased)	−0.0345	−0.0988 to 0.0297	0.290			
Protocol biopsy (yes vs. no)	−0.0820	−0.1479 to − 0.0160	0.015	−0.0815	−0.1461 to 0.0168	0.014
2-year eGFR	−0.0028	−0.0048 to − 0.0008	0.006	−0.0025	−0.0045 to − 0.0006	0.011
Variation in drug level (CV%)	0.0027	0.0004 to 0.0050	0.022	0.0028	0.0005 to 0.0050	0.018
Rejection	0.0870	0.0021 to 0.1720	0.045			

CI, confidence interval; CV%, percent coefficient; eGFR, estimated glomerular filtration rate.

graft biopsy. A total of 13 patients in the PB group (19.12%) and 15 in the NPB group (12.30%) were confirmed to have GN and other diseases (Fig. 5). IgA nephropathy was the leading cause of GN in both groups, which was followed by focal segmental glomerulosclerosis and membranoproliferative GN. In the PB group, about two-thirds (9/13, 69.23%) of the diagnoses were established by protocol biopsy, which provided an opportunity to control the diseases at the subclinical stage.

Survivals beyond 5 years after protocol biopsy

Based on the results, the PB group had higher graft survival 5 years after the protocol biopsy than the NPB group. To test whether the benefit is eternal, we compared the rejection-free and death-censored graft survival 10 years after protocol biopsy between the groups. The overall rejection rate (including BL-R and rejection) was similar between the PB and NPB groups 10 years after the 2-year protocol biopsy (20.64% vs. 18.23%; Fig. 6a). Four grafts in the PB group were lost 6 years after protocol biopsy due to rejection. Death-censored graft survival 10 years after protocol biopsy was 89.00% and 85.62% in PB and NPB groups, respectively ($P = 0.2886$). Subgroup analysis of living donor recipients showed similar results (PB vs. NPB: 88.11% vs. 70.79%, $P = 0.072$, Fig. S3).

Discussion

In this retrospective study, we demonstrated that 2-year protocol biopsy has a positive rate of approximately 20%

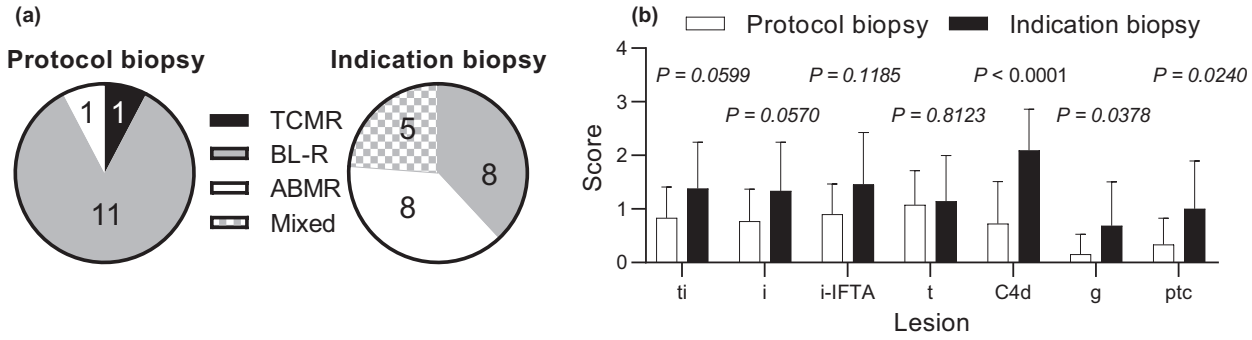

Figure 4 Comparison of rejection results between the protocol and indication biopsies. Both biopsies have different distributions of rejection types (a) and Banff scores (b)

for subclinical or borderline rejection in clinically stable patients. Its detection rate is higher than that of short-term protocol biopsy (i.e., within 1 year), which is usually <10% in the era of tacrolimus and mycophenolate mofetil according to the literature [6,7,13]. Because of its low yield rate, short-term protocol biopsy may not contribute to the improvement of renal function or graft survival despite aggressive treatment after protocol biopsy [6]. Medication noncompliance remains one of the most significant causes of late rejection [2]. In the first year after transplantation, most patients would adhere to the medication regimen; thereafter, some tend to miss their medications. In addition, transplant doctors would intend to lower the dosage of immunosuppressants when renal function remains stable in order to minimize side effects. A protocol biopsy at the end of the second year helps to identify stable patients and those with early rejection, which in turn allows treatment of the latter for renal function preservation.

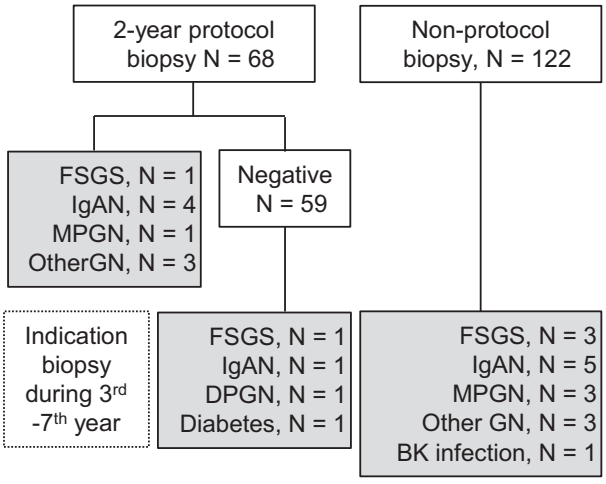

Figure 5 Biopsy results besides rejection. Both protocol and indication biopsies revealed other renal pathologies, which are mainly different types of glomerulonephritis. DPGN, diffuse proliferative glomerulonephritis; FSGS, focal segmental glomerulosclerosis; GN, glomerulonephritis; IgAN, IgA nephropathy; MPGN, membranoproliferative glomerulonephritis

In this study, BL-R is the major type of rejection based on the 2-year protocol biopsy (11/13), and only one case of ABMR was noted. In indication biopsy, the rejection type distribution was variable, and ABMR was found in more than half of all patients [12/21 (eight pure ABMR and four mixed rejections); Fig. 2]. Moreover, in the PB group, all three patients with ABMR by event biopsy had BL-R or TCMR, as determined by protocol biopsy. This result corresponds to previous clinical observations that early subclinical rejection is a risk factor for late ABMR [14–16], which is the major cause of graft failure [2]. The superior graft survival in the PB group compared with that in the NPB group could be attributed to the early detection and treatment of subclinical rejection that certain parts of late rejection were prevented.

No clinical guideline on whether BL-R should be treated or not has been established [17–19]. BL-R has a broad and ambiguous definition; thus, not all diagnoses of BL-R have a direct relationship with rejection by clinical consequence or molecular phenotyping [20,21]. Nevertheless, patients with BL-R with impaired renal function could not recover if left untreated [22]. A recent study by Nankivell et al. [23] showed that both borderline and subclinical rejection episodes have an increased risk of ABMR and graft failure, although 65.8% of patients with BL-R in this study were treated with different therapies. Another study by Zachariah et al. [24] revealed that BL-R identified by protocol biopsy 1 year after transplantation and treated only by adjusting immunosuppressants without pulse steroids is a significant factor for long-term functional decline. Moreover, both naïve and memory donor-specific antibody responses are CD4+ T cell-dependent [25], so an adequate control of T-cell activation

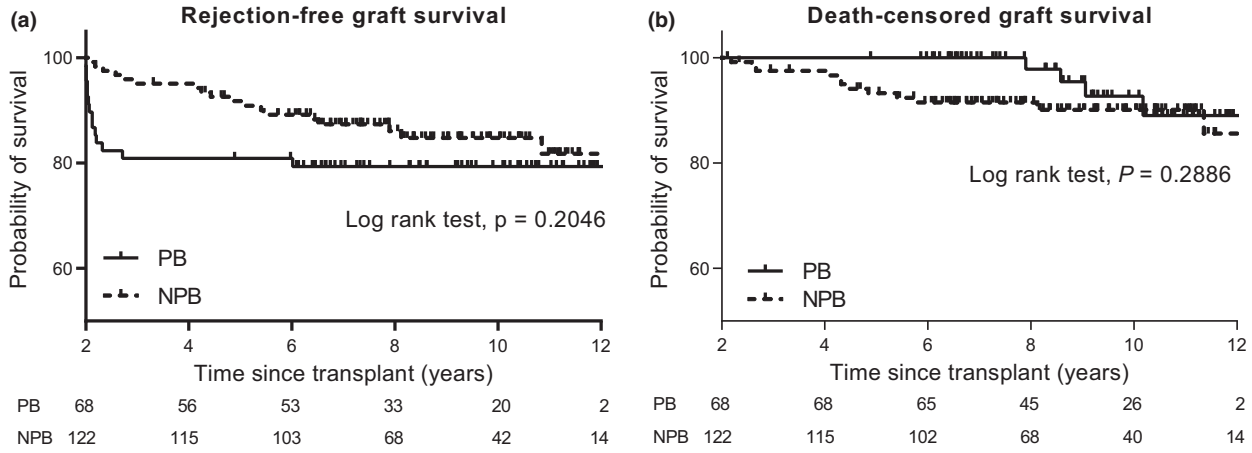

Figure 6 Comparison of long-term survival (12 years) between the two study groups. Kaplan–Meier curves were plotted for rejection-free (a) and death-censored (b) graft survivals

is essential for preventing ABMR. Hence, although not all patients with BL-R are undergoing rejection, we treated all of them as definite rejection in our study, and some of the patients with BL-R in the PB group were possibly overtreated with unnecessary pulse steroid therapy. Nevertheless, under close surveillance, no patient had infection after the treatment, and no graft loss was noted in the subsequent 5 years with fewer diagnoses of ABMR in the PB group.

In addition to rejection, recurrent or de novo renal diseases, such as GN, are associated with graft injury and failure [26–28]. The recurrence time and rate of GN after transplantation depends on its type. In our cohort, IgA nephropathy was the most prevalent GN type in both the PB and NPB groups, which is consistent with previous reports [29,30]. With 2-year protocol biopsy, it is possible to detect early recurrence of GN before the presence of impaired renal function, proteinuria, and hematuria. Although specific therapeutic choices for recurrent GN are limited, patients may receive interventions that could help improve graft survival, including immunosuppression optimization, CD20 antibody administration, and plasmapheresis [30,31], before graft function deterioration. In our study, no graft loss related to GN was noted in the PB group, whereas two patients lost their grafts because of IgA nephropathy in the NPB group. However, the number of patients in our study was too small to draw a conclusion. Thus, further investigation is needed.

Previous studies on serial long-term protocol biopsy [32,33] showed that the incidence of major histologic injury increased from 5% at implantation to 82% at 10 years. Both immunologic and nonimmunologic causes induce graft injury, resulting in late graft loss. In this study, we also observed that 5 years after protocol biopsy, the graft survival benefits diminished due to late rejection in the PB group. Ten years later, the difference in death-censored graft survival between the PB and NPB groups became nonsignificant. We could not draw a definitive conclusion regarding this observation due to the relatively small sample size. Nevertheless, examining the four graft losses, all of them could be attributed to rejection. Two had positive findings of rejection, while the other two had negative findings by protocol biopsy. After protocol biopsy, the effect of treatment could be insufficient or wean off over time, and a negative biopsy result at 2 years does not necessarily represent good long-term survival. In clinical practice, we also found that medication compliance would change in some patients after a long period of stable allograft function with favorable biopsy results. Maintenance of allograft function is affected by multiple dynamic factors, including immunosuppression, autoimmune status, environment, and aging. Hence, a single protocol biopsy at 2 years helps to detect early histologic changes that require intervention but does not guarantee long-term graft survival at 10 years. We postulated that de novo or recurrent rejection could develop 2–3 years after protocol biopsy, which may continue to induce graft damage. Thus, repeated protocol biopsy in $\leq$5 years may be needed for better surveillance and to preserve the advantage of protocol biopsy.

This study has some limitations. This was a single-center retrospective study. Only one-third of the patients underwent protocol biopsy, and their participation was according to their will. Although each patient had different concerns, a selection bias was possible; nonadherence to medical treatment could be associated with the

patients' response to the clinical proposition. We checked the variation in drug level, which is a predictive factor for graft failure by multivariate analysis, and found no significant difference between the PB and NPB groups, which means medication nonadherence might not be related to the decision of accepting the proposition of protocol biopsy in our study. In our center, some patients refused to undergo protocol biopsy, and the most common reason was to avoid putting the graft at risk instead of noncompliance. Since no strong evidence for the benefits of protocol biopsy exists, we could only propose it to the patient; thus, we conducted this study. Furthermore, demographic profiles showed that the patients were younger, and the proportion of living donors was higher in the PB group, which could be attributed to the selection bias of the PB and NPB group. According to the database of the United Network for Organ Sharing and several clinical reports, younger recipients and organs from older donors have a higher rejection rate [34,35]. However, such trends were not observed in our study, which could be because of the effect of protocol biopsy. Recipient age is not a risk factor for poor allograft survival [36]; rather, younger patients may be more open to the proposition of protocol biopsy. Moreover, renal grafts from living donors have better survival rates [37,38], which could be cofounding in the graft survival in the PB group. Nevertheless, organ survival differs between living and deceased donors, mainly in the first year after transplantation because of the uncontrolled condition of the organs from deceased donors. Therefore, our study has chosen stable patients at 2 years to reduce the effect of the organ from deceased donors. In the subgroup analysis, we also showed that living donor recipients in the PB group have the same 5-year survival benefits after 2-year protocol biopsy compared to the NPB group (Fig. S3).

Protocol biopsy is not a standard-of-care procedure in most centers, and no guideline for appropriate timepoints has been established. In this study, we have shown that in selected patients with stable renal graft conditions, 2-year protocol biopsy provides an opportunity to detect early histopathological changes. Patients who had protocol biopsy and received aggressive treatment for rejection had higher graft survival 5 years after the protocol biopsy than those without protocol biopsy; however, further surveillance may be needed to maintain this benefit. Protocol biopsy is a simple and feasible strategy for most centers to improve long-term outcomes.

Authorship

C-CC and M-KT: participated in research design. C-CC, W-CL, C-YL and M-KT: participated in the writing of the paper. C-CC, W-CL, C-YY and M-KT: participated in the performance of the research. C-CC and C-YL: participated in data analysis.

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Conflicts of interest

The authors have declared no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Biopsy results in the excluded patients with preformed DSA and ABO incompatibility.

Figure S2. Biopsy results in the excluded patients who underwent indication biopsy within 2 years.

Figure S3. Comparison of graft survival for living donor recipients.

Table S1. Patients with malignancy after kidney transplantation.

Table S2. Histologic findings of biopsies evaluated using Banff scores.

REFERENCES

1. Lamb KE, Lodhi S, Meier-Kriesche H-U. Long-term renal allograft survival in the United States: a critical reappraisal. *Am J Transplant* 2011; **11**: 450.
2. Sellarés J, de Freitas DG, Mengel M, *et al*. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. *Am J Transplant* 2012; **12**: 388.

3. Loupy A, Lefaucheur C. Antibody-mediated rejection of solid-organ allografts. *N Engl J Med* 2018; **379**: 1150.
4. Ekberg H, Tedesco-Silva H, Demirbas A, *et al*. Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007; **357**: 2562.
5. Ekberg H, Bernasconi C, Tedesco-Silva H, *et al*. Calcineurin inhibitor minimization in the symphony study: observational results 3 years after transplantation. *Am J Transplant* 2009; **9**: 1876.
6. Rush D, Arlen D, Boucher A, *et al*. Lack of benefit of early protocol biopsies in renal transplant patients receiving TAC and MMF: a randomized study. *Am J Transplant* 2007; **7**: 2538.
7. Gloor JM, Cohen AJ, Lager DJ, *et al*. Subclinical rejection in tacrolimus-treated renal transplant recipients. *Transplantation* 2002; **73**: 1965.
8. Muramatsu M, Miyagi M, Ishikawa Y, *et al*. Baseline glomerular sclerosis influences morphological changes, but not level of serum creatinine. *Transplant Proc* 2005; **37**: 709.
9. Mehta R, Cherikh W, Sood P, Hariharan S. Kidney allograft surveillance biopsy practices across US transplant centers: a UNOS survey. *Clin Transplant* 2017; **31**: e12945.
10. Cosio FG, El Ters M, Cornell LD, Schinstock CA, Stegall MD. Changing kidney allograft histology early posttransplant: prognostic implications of 1-year protocol biopsies. *Am J Transplant* 2016; **16**: 194.
11. Yang C-Y, Lee C-Y, Yeh C-C, Tsai M-K. Renal transplantation across the donor-specific antibody barrier: graft outcome and cancer risk after desensitization therapy. *J Formos Med Assoc* 2016; **115**: 426.
12. Hsiau M, Fernandez HE, Gjertson D, Ettenger RB, Tsai EW. Monitoring nonadherence and acute rejection with variation in blood immunosuppressant levels in pediatric renal transplantation. *Transplantation* 2011; **92**: 918.
13. Nankivell BJ, Borrows RJ, Fung CL-S, O'Connell PJ, Allen RDM, Chapman JR. Natural history, risk factors, and impact of subclinical rejection in kidney transplantation. *Transplantation* 2004; **78**: 242.
14. Gloor JM, Sethi S, Stegall MD, *et al*. Transplant glomerulopathy: subclinical incidence and association with alloantibody. *Am J Transplant* 2007; **7**: 2124.
15. Moreso F, Carrera M, Goma M, *et al*. Early subclinical rejection as a risk factor for late chronic humoral rejection. *Transplantation* 2012; **93**: 41.
16. Wiebe C, Gibson IW, Blydt-Hansen TD, *et al*. Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant. *Am J Transplant* 2012; **12**: 1157.
17. Beimler J, Zeier M. Borderline rejection after renal transplantation–to treat or not to treat. *Clin Transplant* 2009; **23**(Suppl 21): 19.
18. Haas M, Loupy A, Lefaucheur C, *et al*. The Banff 2017 kidney meeting report: revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. *Am J Transplant* 2018; **18**: 293.
19. Becker JU, Chang A, Nickeleit V, Randhawa P, Roufosse C. Banff borderline changes suspicious for acute T cell-mediated rejection: where do we stand? *Am J Transplant* 2016; **16**: 2654.
20. de Freitas DG, Sellarés J, Mengel M, *et al*. The nature of biopsies with "borderline rejection" and prospects for eliminating this category. *Am J Transplant* 2012; **12**: 191.
21. Reeve J, Chang J, Salazar IDR, Lopez MM, Halloran PF. Using molecular phenotyping to guide improvements in the histologic diagnosis of T cell-mediated rejection. *Am J Transplant* 2016; **16**: 1183.
22. Palomar R, Ruiz JC, Val-Bernal F, *et al*. Borderline changes in kidney transplantation: evolution of treated cases versus nontreated. *Transplant Proc* 1999; **31**: 2314.
23. Nankivell BJ, Agrawal N, Sharma A, *et al*. The clinical and pathological significance of borderline T cell-mediated rejection. *Am J Transplant* 2019; **19**: 1452.
24. Zachariah MS, Dwivedi AK, Yip CS, *et al*. Utility of serial protocol biopsies performed after 1 year in predicting long-term kidney allograft function according to histologic phenotype. *Exp Clin Transplant* 2018; **16**: 391.
25. Chen C-C, Koenig A, Saison C, *et al*. CD4+ T cell help is mandatory for naive and memory donor-specific antibody responses: impact of therapeutic immunosuppression. *Front Immunol* 2018; **9**: 275.
26. Naesens M, Kuypers DRJ, De Vusser K, *et al*. The histology of kidney transplant failure: a long-term follow-up study. *Transplantation* 2014; **98**: 427.
27. Choy BY, Chan TM, Lai KN. Recurrent glomerulonephritis after kidney transplantation. *Am J Transplant* 2006; **6**: 2535.
28. Ponticelli C, Glassock RJ. Posttransplant recurrence of primary glomerulonephritis. *Clin J Am Soc Nephrol CJASN* 2010; **5**: 2363.
29. Furness PN, Roberts ISD, Briggs JD. Recurrent glomerular disease in transplants. *Transplant Proc* 2002; **34**: 2422.
30. Cosio FG, Cattran DC. Recent advances in our understanding of recurrent primary glomerulonephritis after kidney transplantation. *Kidney Int* 2017; **91**: 304.
31. Lim WH, Shingde M, Wong G. Recurrent and de novo glomerulonephritis after kidney transplantation. *Front Immunol* 2019; **10**: 1944.
32. Stegall MD, Park WD, Larson TS, *et al*. The histology of solitary renal allografts at 1 and 5 years after transplantation. *Am J Transplant* 2011; **11**: 698.
33. Stegall MD, Cornell LD, Park WD, Smith BH, Cosio FG. Renal allograft histology at 10 years after transplantation in the tacrolimus era: evidence of pervasive chronic injury. *Am J Transplant* 2018; **18**: 180.
34. Tullius SG, Milford E. Kidney allocation and the aging immune response. *N Engl J Med* 2011; **364**: 1369.
35. Colvin MM, Smith CA, Tullius SG, Goldstein DR. Aging and the immune response to organ transplantation. *J Clin Invest* 2017; **127**: 2523.
36. Fabrizii V, Winkelmayer WC, Klauser R, *et al*. Patient and graft survival in older kidney transplant recipients: does age matter? *J Am Soc Nephrol JASN* 2004; **15**: 1052.
37. Terasaki PI, Cecka JM, Gjertson DW, Takemoto S. High survival rates of kidney transplants from spousal and living unrelated donors. *N Engl J Med* 1995; **333**: 333.
38. Axelrod DA, McCullough KP, Brewer ED, Becker BN, Segev DL, Rao PS. Kidney and pancreas transplantation in the United States, 1999–2008: the changing face of living donation. *Am J Transplant* 2010; **10**(4 Pt 2): 987.