The Protective Role of Protocol Biopsy for Allograft Kidney Maintenance in Kidney Transplantation

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Abstract

Many studies have reported that protocol biopsy (PB) may help preserve kidney function in kidney transplant recipients. Early detection and treatment of subclinical rejection may reduce the incidence of chronic allograft nephropathy and graft failure. However, no consensus has been reached regarding PB effectiveness, timing, and policy. This study aimed to evaluate the protective role of routine PB performed 2 weeks and 1 year after kidney transplantation. We reviewed 854 kidney transplant recipients at the Samsung Medical Center between July 2007 and August 2017, with PBs planned at 2 weeks and 1 year after transplantation. We compared the trends in graft function, chronic kidney disease progression, new-onset chronic kidney disease, infection, and patient and graft survival between the 504 patients who underwent PB and 350 who did not undergo PB. The PB group was again divided into two groups: the single PB group (n = 207) and the double PB group (n = 297). In the PB group, the donors and recipients were significantly older and there was a greater presence of recipient diabetes mellitus and donor hypertension, donor-specific antigen, and a higher proportion of ABO-incompatible kidney transplantations. The PB group was significantly different from the no-PB group in terms of the trends in graft function (estimated glomerular filtration rate). The Kaplan-Meier curve showed that PB did not significantly improve graft survival or overall patient survival. However, in the multivariate Cox analysis, the double PB group had advantages in graft survival, chronic kidney disease progression, and new-onset chronic kidney disease. PB can play a protective role in the maintenance of kidney grafts in kidney transplant recipients.

Introduction

Kidney transplantation (KT) has been shown to result in longer patient survival compared to dialysis and is now the treatment of choice for chronic kidney disease (CKD)¹. In addition, cost and quality of life are better with KT compared with dialysis, though graft management is essential for long-term maintenance^{2,3}.

Therefore, proper management strategies to maintain long-term graft survival are necessary. One strategy that many institutions have adopted is protocol biopsy (PB) with follow-up of transplant recipients^{4,5}. As histological diagnosis is the most effective modality for graft evaluation, the primary goal of PB is the early detection and treatment of subclinical rejection (SCR), which is characterized by tubule-interstitial infiltration of the renal allograft without clinical deterioration^{6,7}.

SCR diagnosed by PB is associated with chronic allograft nephropathy, which is the most common cause of allograft failure in KT⁸⁻¹¹. Furthermore, chronic allograft nephropathy and graft survival are strongly correlated with acute rejection episodes during the first year after renal transplantation¹²⁻¹⁵; therefore, the early detection and treatment of SCR might reduce the progression of chronic allograft nephropathy and improve graft survival^{8,16,17}. PBs can also be used to evaluate the baseline status of donor renal grafts

and to detect BK polyomaviral (BKV) nephropathy, calcineurin inhibitor nephrotoxicity, interstitial fibrosis, and tubular atrophy early^{18,19}.

In our institution, routine PBs are performed 2 weeks and 1 year after KT. Routine PBs are implemented to detect SCR early and administer high-dose corticosteroid pulse therapy to appropriately manage the risk of chronic tubule-interstitial damage. In addition, routine PBs are performed to detect primary disease recurrence (e.g., immunoglobulin A nephropathy, focal segmental glomerulosclerosis, diabetic nephropathy), to proceed with appropriate treatment.

Therefore, this study aimed to evaluate the protective effects of PB by comparing renal allograft function (estimated glomerular filtration rate [eGFR]), CKD progression, new-onset CKD, and graft and patient survival before and after the application of routine PB.

Methods

Study Population. From July 2007 to August 2017, 1,361 KTs were performed at the Samsung Medical Center (Fig. 1). Patients with dual kidney or multi-organ transplants (including simultaneous liver grafts), re-transplantation and pediatric transplants were excluded from the study (n = 337). Additionally, patients who transplanted during the PB strategy period but did not undergo PB, and those diagnosed with SCR but who were not treated, were also excluded (n = 170). The remaining 854 eligible patients were included in the study.

After enrollment, the patients were divided into two groups: the no-protocol biopsy (nPB) strategy period group (July 2007 to July 2012; n = 350) and the PB strategy period group (August 2012 to August 2017; n = 504). The PB group was then divided into two additional groups: the single protocol biopsy (sPB) group, for which PB was performed once at 2 weeks or 1 year after transplantation (n = 207), and the double protocol biopsy (dPB) group, for which PB was performed both at 2 weeks and 1 year after transplantation (n = 297).

After group classification, we compared the trends in graft function, CKD progression, new-onset CKD, infection, and patient and graft survival among the groups (nPB vs. PB and nPB vs. sPB vs. dPB).

Protocol biopsy and treatment of rejection. PBs were performed percutaneously under real-time ultrasonographic guidance at 2 weeks and 1 year after transplantation. Pathologic evaluation of the biopsies according to the Banff criteria of that period was performed by our transplant pathologists²⁰. For our treatment strategy, a borderline pathologic change is considered acute rejection and requires treatment. Clinical rejection was defined as the diagnosis with indicational biopsy, which is performed when the patient shows suspicious finding of acute rejection such as elevation of serum creatinine level or new onset proteinuria. SCR was defined as the findings of a protocol biopsy performed without any suspicion of acute rejection. Both Clinical rejection and SCR were included in the rejection episode analysis.

When patients were diagnosed with acute cellular rejection (ACR), steroid therapy was initiated. Intravenous methylprednisolone 500 mg/day for 3 days, tapered by half every day to 60 mg/day was the initial steroid regimen. Oral methylprednisolone was then initiated at 32 mg/day and then tapered to 4–8 mg/day within 1–2 weeks and to 4 mg/day for maintenance. For PB-confirmed antibody-mediated rejection (AMR), intravenous immunoglobulins were administered, or plasmapheresis was performed.

Immunosuppression protocol. Depending on the routine induction medication, rabbit antithymocyte globulin (rATG) or basiliximab were administered. In living donor KT, rATG was administered at 1.5 mg/kg/day from the time of surgery to postoperative day 2, in positive human leukocyte antigen (HLA) crossmatch, donor-specific antigen (DSA) with a mean fluorescence intensity $\geq 2,500$, and ABO-incompatible KT. In deceased donor KT, rATG was administered in extended criteria donor KT. Otherwise, basiliximab was administered at a dose of 20 mg/day at the time of surgery and on postoperative day 4.

All patients received triple immunosuppressive therapy regimens consisting of tacrolimus, mycophenolate mofetil, and methylprednisolone. Any patient who did not receive this regimen was excluded from the study. Tacrolimus (FK506, Prograf; Astellas Fujisawa, Osaka, Japan, and generic tacrolimus) was started on postoperative day 1 at 0.1–0.15 mg/kg/day and adjusted to maintain whole-blood trough levels at 8–10 ng/mL for 1–2 months postoperatively and at 6–8 ng/mL thereafter. Mycophenolate mofetil (Myfortic; Novartis Pharma AG, Basel, Switzerland) was started at a dose of 540 mg/day on postoperative day 1 and adjusted according to the white blood cell count. Methylprednisolone was started on the day of surgery at an intravenous dose of 500 mg/day and administered for 2 days and then tapered by half every day to 60 mg/day. Thereafter, oral methylprednisolone was administered at 32 mg/day for 7 days, 16 mg/day for the next 2 weeks, 8 mg/day for the next month, and 4 mg/day for maintenance.

Prophylaxis of infectious diseases. Our protocol for the prophylaxis and diagnosis of infectious diseases was uniform throughout the study period. All patients underwent routine screening for cytomegalovirus (CMV) antigenemia, and recipients with rATG induction received intravenous ganciclovir prophylaxis at 5 mg/kg/day for 2 weeks. Screening for polyomavirus infection was based on routine urinary cytology testing followed by plasma polymerase chain reaction for BKV DNA and graft biopsy.

All patients also received 1 tab/day of trimethoprim/sulfamethoxazole (TMP/SMX, 80 mg TMP/400 mg SMX) for *Pneumocystis jirovecii* prophylaxis. As preemptive prophylaxis for fungal and viral infections, TMP/SMX was administered for up to 6 months after KT in all patients, and itraconazole was additionally administered for up to 2 weeks for patients who underwent rATG induction. After steroid pulse therapy, TMP/SMX was administered for 6 weeks.

Definition of the variables. The primary outcome assessed in this study was the inter-stage progression of CKD, with a > 25% decrease in the eGFR. The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation, and patients were assigned to a CKD stage based on the Kidney

Disease: Improving Global Outcomes guidelines (KDIGO guidelines)²¹. CKD inter-stage progression was calculated using all measured outpatient creatinine values and consecutive 6-month intervals. Stage progression was defined as a significant enough decrease in the mean eGFR in a given 3-month block to result in the inter-stage progression of CKD. If no values were obtained during the block, no progression was considered to have occurred. Pretransplant renal function was determined and initially assessed for its potential effect on disease progression on 1-year post-operation. Since disease progression was based on renal function at 1-year post-transplantation, we did not analyze the pretransplant renal function. New-onset CKD was defined in patients with an eGFR of 60 mL/min/1.73 m² or higher who were not diagnosed with CKD. Utilizing the KDIGO guidelines for eGFR, new-onset CKD was defined as an eGFR < 60 mL/min/1.73 m² (GFR categories G3a–G5) lasting more than 3 months.

Post-KT glomerulonephritis was defined as newly diagnosed or recurrent immunoglobulin A nephropathy, focal segmental glomerulosclerosis, or diabetic nephropathy, and viral infection was defined as influenza or zoster virus infection (not including CMV and BKV).

Statistical analysis. All variables are expressed as the mean ± standard deviation or number and percentage. Between-group differences for continuous variables were compared using the Mann-Whitney test, and between-group differences for numbers and percentages were compared using the χ^2 test or Fisher's exact test. Kaplan-Meier survival curves were used to estimate CKD progression, new-onset CKD, and graft and overall survival. A linear mixed model was used to analyze the difference in the trend of graft function among the three groups. Cox regression analysis was performed with adjustments for significant risk factors in the univariate analysis, as well as other factors previously known to be associated with CKD progression, new-onset CKD, and graft survival. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using IBM SPSS Statistics for Windows (version 25.0; SPSS, Inc., Chicago, IL, USA).

Results

Baseline characteristics. The PB group had a significantly higher donor and recipient age, the proportion of males, presence of recipient diabetes mellitus (DM), and donor hypertension than the nPB group (Table 1). However, there was no significant difference between the sPB and dPB groups. Additionally, there was no difference in donor type between the PB and nPB groups. However, when the three groups were compared, there were more deceased donors in the sPB group, which remained true when comparing the sPB and dPB groups. High HLA II mismatch KT, the presence of DSA, ABO-incompatible KT, cold ischemic time, and warm ischemic time were higher in the PB group, but there were no significant differences between the sPB and dPB groups. In the PB group, rATG was used more frequently as an induction regimen.

Among all patients, the number of rejection episodes was higher in the PB group, but the proportion of clinical rejection was higher in the nPB group. Moreover, post-KT glomerulonephritis, CKD progression, new-onset CKD, and graft failure were higher in the nPB group (Table 2). Comparing the two groups,

clinical rejection and rejection episodes were higher in the dPB group, but CKD progression and new-onset CKD were lower in the dPB group, and graft failure and mortality rates were significantly lower in the sPB group.

Trends in graft function and CKD progression. Graft function was analyzed using eGFR trends. Comparing the two groups (PB vs. nPB) and the three groups (nPB vs. sPB vs. dPB), there was a significant difference in the eGFR trends (Fig. 2). Graft function, in general, was lower in the early period after the transplant and improved around 1 year after KT, but was better maintained in the PB group, especially in the dPB group.

There was a significant difference in CKD progression between the PB and nPB groups and between the nPB and dPB groups according to the Kaplan-Meier curve (Fig. 3). In the Cox regression analysis of CKD progression, donor DM, and post-KT glomerulonephritis as risk factors, rejection episodes were significantly associated with CKD progression, but dPB and sPB played a significant protective role in CKD progression (Table 3).

There was no significant difference in new-onset CKD between the PB and nPB groups according to the Kaplan-Meier curve; however, in the three-group analysis, there was a significant difference between the dPB and the other groups (Fig. 4). In the Cox regression analysis of new-onset CKD, high HLA I mismatch, and post-KT glomerulonephritis as risk factors, rejection episodes were significantly associated with new-onset CKD, and dPB played a significant protective role in new-onset CKD (Table 4).

Graft survival and infection outcomes. In the graft survival analysis using the Kaplan-Meier curve, no significant difference was observed between the two groups (Fig. 5). In the multivariate Cox regression analysis of graft survival, recipient DM, and post-KT glomerulonephritis as risk factors, rejection episodes were significantly associated with poor prognosis, but dPB played a significant protective role in graft survival (Table 5).

In terms of the number of infections up to 2 years after KT, the PB group had more fungal, viral, and CMV infections than the nPB group, but there was no difference between the sPB and dPB groups (Table 2). In the PB group, almost all patients recovered; however, there were 8 infection-related deaths (bacterial, 3; fungal, 4; and viral, 1).

The most common cause of death was multi-organ failure associated with sepsis resulting from urinary tract infection and pneumonia. In the multivariate Cox regression analysis, there was no significant difference between CMV and BKV infections for predicting CKD progression, new-onset CKD, or graft survival (Tables 3-5).

Discussion

Due to donor organ shortage, the transplant graft must be properly managed to maintain its function over time; therefore, periodic laboratory examinations are conducted to monitor graft function. However,

laboratory data alone is not sufficient to determine the condition of the graft. Therefore, PB is a useful diagnostic tool for identifying lesions, predicting the risk of graft failure, and guiding future directions for both organ recipients and donors²².

However, a survey of US kidney transplant centers found that only 17% perform PBs routinely on all transplant recipients, and studies show that noninvasive methods such as fibroscans or the evaluation of biological markers are effective at detecting SCR^{23,24}. Moreover, PBs are inconvenient for patients, carry a risk for complications, are invasive and costly, and require technical expertise^{25–27}. Therefore, there is no current consensus on the timing and method of PBs, or their effectiveness²⁸. In addition, consensus regarding appropriate guidelines for PB has not yet been established, and while some studies on PBs have been performed, only a small number of enrolled patients have been included^{8,19,28}.

As previously mentioned, the current policy at our institution is to conduct PB twice, once at 2 weeks and again 1 year after transplantation, because rejection that occurs soon after transplantation is considered a major risk factor for chronic rejection and graft failure. In addition, interstitial fibrosis and tubular atrophy are present in approximately half of all grafts with stable function by 1 year after transplantation¹⁵.

This study demonstrated the protective role of PB in KT. The trend of graft function preservation was more apparent in the PB group than in the nPB group, and PB was associated with significant protective effects on CKD progression and new-onset CKD. This is likely because SCR was detected and treated relatively early in this group, which is thought to lead to better results. In addition, dPB had more significant results than sPB in terms of new-onset CKD. Therefore, PB may have advantages over nPB, and dPB may be superior to sPB.

PB is invasive and carries a small but real risk of complications, with the best estimates for major complications between 0.4% and 1.0%^{27,29}. In this study, there were no complications requiring intervention after PB. However, from 2012 to 2019, the rate of major complications from PB at the Samsung Medical Center was 0.45% (4/882) at 2 weeks and 0.17% (1/556) at 1-year post-transplantation in 1,438 KT recipients (yet unpublished data). This is consistent with previous studies and should not limit the application of PB.

PB is an evaluative strategy that can be used to accurately diagnose and monitor for rejection, but steroid pulse therapy after PB can be a risk factor for viral infection. Theoretically, this could lead to worse results in those undergoing PB. In terms of infection outcomes in this study, the PB group had more fungal, viral, and CMV infections than the nPB group, which was likely the result of steroid pulse therapy. The mortality rate from infections for all patients in the PB group was 0.60% bacterial (3/504), 0.79% fungal (4/504), and 0.20% viral (1/504). However, when comparing the patients diagnosed with infections, the mortality rate was significantly higher with fungal infections (4/14; 28.57%) compared to bacterial (3/88; 3.41%) or viral (1/10; 10.00%) infections. A study at our institution has identified that treatments for rejection could be a risk factor for invasive pulmonary aspergillosis-associated mortality³⁰.

However, in the current study, no relationship was found between patient mortality and rejection treatment. This is likely because no infection occurred during the treatment of SCR diagnosed by PB. Indeed, at least one year had elapsed between the treatment of SCR and death, and the cause of death was the rapid progression of infection, likely due to immunosuppression. Therefore, the treatment of SCR diagnosed by PB was not considered a risk factor for infection-related mortality in patients diagnosed with infections. Additionally, the CMV infection rate was also high; however, unlike previous studies, graft survival was not affected. This may be because at our center, ganciclovir is administered as preemptive prophylaxis with periodic CMV monitoring. However, to reach a consensus concerning the prevention of life-threatening infections in KT recipients, more extensive study is needed.

In our study, there was no significant difference in the survival rate based on the Kaplan-Meier curve for graft survival. This is thought to be the result of specific characteristics of the donors and recipients. The PB group included donors and recipients who were significantly older and there was a higher presence of recipient DM and donor hypertension. Therefore, the eGFRs within 1 year after KT were lower in PB group as shown in Fig. 2. Moreover, a number of mismatched HLA II, a presence of DSA, and longer cold and warm ischemic times, which are known risk factors for graft failure, were higher in the PB group^{17,31}. Owing to these differences in baseline characteristics, no significant differences in graft survival trends were observed. However, in the multivariate analysis for graft failure, the Cox regression model showed statistically significant results for the dPB group. Therefore, we concluded that performing PB twice at these time points can play a protective role in the management of kidney grafts.

There were some limitations to this study. First, it was a retrospective, single-center analysis of a cohort in South Korea. Therefore, the results might not be generalizable to other countries and continents. Second, the nPB group included patients who had undergone KT in the relatively recent past; although most conditions were adjusted for, poor results may have resulted from adverse conditions in the medical environment. Third, the prevalence of SCR is higher in the early period after transplantation and falls to low levels by 1 year after KT; therefore, the sPB and dPB groups may not have been homogenous. Next, the patients in the sPB group may have been in a relatively poorer condition. We could not strictly analyze the reasons patients received PB only once, but bleeding tendency and use of anticoagulation medication for cardiovascular disease were some common reasons. Finally, ACR and AMR were not measured separately because most rejections were ACR. For reference, at our center, the rate of ACR is 14.8% and the AMR is 1.7% at 2 weeks post-transplantation, and the ACR is 30.8% and the AMR is 1.8% at 1-year post-transplantation.

In conclusion, PBs can play a protective role in graft kidney maintenance in KT recipients. Rather than a single PB, managing SCR through more active biopsy implementation within 1 year of KT is recommended since the dPB group showed significant protective effects for CKD progression, new-onset CKD, and patient survival in this study. The results of this study indicated that PB with SCR treatment can be a more feasible and safe option for kidney graft evaluation since the risk of complications and life-threatening infections was low. A large-scale, prospective, randomized study is needed to further

investigate the long-term outcomes of PB. With the careful and definitive evaluation of PB, we expect increased graft survival and excellent patient prognosis.

Abbreviations

Protocol biopsy **PB**

Kidney transplantation **KT**

Chronic kidney disease **CKD**

Subclinical rejection **SCR**

BK polyomavirus **BKV**

Glomerular filtration rate **GFR**

Estimated glomerular filtration rate **eGFR**

Acute cellular rejection **ACR**

Antibody-mediated rejection **AMR**

Human leukocyte antigen **HLA**

Donor-specific antigen **DSA**

Rabbit antithymocyte globulin **rATG**

Cytomegalovirus **CMV**

Trimethoprim/sulfamethoxazole **TMP/SMX**

Kidney disease: improving global outcomes **KDIGO**

Diabetes mellitus **DM**

Declarations

Ethical approval and informed consent. This retrospective study was approved by the Institutional Review Board of Samsung Medical Center (No. 2020-11-013), and the need for informed consent was waived. All methods were carried out in accordance with Declaration of Helsinki.

Author contributions

O.L., K.W.L., and J.B.P. participated in the study design, data analysis, data interpretation, and article writing. J.E.L. and K.K. participated in the study design, data analysis, and data interpretation. N.Y.H. participated in the data analysis and data interpretation.

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Competing interests

The authors of this manuscript have no conflicts of interest, as described by the Nephrology Dialysis Transplantation, to disclose.

Data availability statement

The data that support the findings of this study are available from corresponding author upon reasonable request.

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Tables

Figures

Figure 1

Flow diagram of patient selection.

Figure 2

Linear mixed model showing the differences in the graft function trends between the two groups (PB vs. nPB) and the three groups (nPB vs. sPB vs. dPB). Graft function was lower in the early period after transplantation and improved around 1 year after KT, and was better maintained in the PB group, especially in the dPB group.

Analysis For CKD progression

Analysis For CKD progression

Figure 3

According to the Kaplan-Meier curve, a significant difference in CKD progression was found between the PB and nPB groups in the two-group analysis and between the nPB and dPB groups in the three-group analysis.

Analysis For New onset CKD

Analysis For New onset CKD

Figure 4

According to the Kaplan-Meier curve, no significant difference in new-onset CKD was found between the PB and nPB groups in the two-group analysis, but a significant difference was found between the dPB and the other groups in the three-group analysis.

Figure 5

According to the Kaplan-Meier curve, no significant difference was found between the PB and nPB groups in terms of graft survival (A) and overall survival (B).

Supplementary Files

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