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Bisphosphonates as a Tacrolimus-Sparing Strategy in Kidney Transplantation: Insights from a Retrospective Analysis

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Purpose: Due to chronic toxicity, tacrolimus-sparing is an important issue in kidney transplant recipients (KTRs). Several studies have shown that bisphosphonate use is associated with favorable graft outcomes in KTRs. We investigated whether the association between tacrolimus trough levels (TTLs) and graft outcomes differed according to bisphosphonate use in KTRs.

Materials and Methods: We conducted a retrospective study encompassing 1441 KTRs who were administered tacrolimus-based immunosuppressants. The primary exposure was a time-dependent cross-product of TTLs (low TTLs vs. normal-high TTLs with a reference of 6 ng/mL) and bisphosphonate use. Two primary outcomes were evaluated: overall graft loss (death or conversion to kidney replacement) and an estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m^2.

Results: During the median follow-up of 6.1 (3.4–9.7) years, overall graft loss occurred in 157 (10.9%) patients. Cox regression revealed that normal-high TTLs without bisphosphonate use were associated with a reduced risk of overall graft loss [adjusted hazard ratio (aHR), 0.65; 95% confidence interval (CI), 0.45–0.95] compared to low TTLs without bisphosphonate use. The use of bisphosphonate in conjunction with normal-high TTLs correlated with an even lower risk of overall graft loss (aHR, 0.25; 95% CI, 0.08–0.80) compared with low TTLs without bisphosphonate use. In patients with low TTLs, bisphosphonate use was associated with a reduced risk of overall graft loss compared with non-use (aHR, 0.20; 95% CI, 0.09–0.43). Similar trends were observed in the eGFR outcome.

Conclusion: The use of bisphosphonate was associated with favorable graft outcomes, even with low TTLs. Incorporating bisphosphonate into a conventional immunosuppressant regimen may potentially reduce tacrolimus requirement.

Key Words: Bisphosphonate, tacrolimus, kidney transplantation, graft outcome

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INTRODUCTION

The introduction of tacrolimus into immunosuppressive regimens for kidney transplants has notably improved graft survival by substantially reducing the incidence of acute rejection.[1] However, chronic allograft nephropathy, partly attributed to calcineurin inhibitor (CNI) toxicity, remains a significant challenge, compromising long-term graft survival.[2] Moreover, even when maintained within the recommended therapeutic range, prolonged CNI exposure has been associated with an increased risk of malignancy and metabolic complications, including new-onset diabetes mellitus and dyslipidemia.[3,4] Consequently, minimizing the adverse effects of immunosuppressive therapies has become a key objective in the long-term management of kidney transplant recipients (KTRs), highlighting the clinical importance of developing novel immunosuppressive strategies that allow for CNI dose reduction.[5,6]

Bisphosphonates, primarily indicated for the treatment of osteoporosis and bone metastasis, exert their therapeutic effects by inhibiting osteoclastic bone resorption. In addition to their role in bone metabolism, bisphosphonates have been shown to exert immunomodulatory effects on both innate and adaptive immune responses.[7] Recently, our research group identified an association between the use of bisphosphonates and favorable long-term graft outcomes in KTRs,[8] supporting findings from another study that demonstrated a positive relationship between ibandronate treatment within the first year post-kidney transplantation and graft survival.[9] Furthermore, bisphosphonate use has been associated with a decreased incidence of acute rejection in KTRs,[10] a finding consistent with prior evidence from animal models of heart, pancreas, and small intestine transplantation.[11-13]

Based on these findings, we hypothesized that bisphosphonates may exert a tacrolimus-sparing effect. To evaluate this hypothesis, we investigated whether the association between tacrolimus trough levels (TTLs) and graft outcomes varied according to the co-administration of bisphosphonates in KTRs.

MATERIALS AND METHODS

Data source

We screened 2236 adult patients who underwent kidney transplantation at Severance Hospital, South Korea, between January 2006 and December 2020. Patients who received tacrolimus continuously throughout the study period, without switching or discontinuation, were included. Patients were excluded if they were treated with cyclosporine or rapamycin, had incomplete tacrolimus data, had a history of preoperative bisphosphonate use, received bisphosphonates for less than 1 year, underwent re-transplantation, or had missing information for transplant-related factors such as donor type, ABO incompatibility, human leukocyte antigen (HLA) mismatch, and year of transplantation. After applying these criteria, a total of 1441 patients were included in the final analysis (Fig. 1). This study was conducted in accordance with the Declaration of Helsinki and the Declaration of Istanbul, and it was approved by the Institutional Review Board of the Yonsei University Health System (IRB No. 2022-2138-001). Informed consent was waived due to the retrospective design.

Bisphosphonate use and bone mineral density

Bisphosphonate use was defined as continuous administration for at least 1 year or more and was assessed monthly using the electronic medical record system at Severance Hospital. The bisphosphonates prescribed included risedronate, ibandronate, alendronate, pamidronate, and zoledronate. Based on the typical prescription interval of each drug (risedronate: 7–30 days; ibandronate: 30–90 days; alendronate: 7 days; pamidronate: 90 days; zoledronate: 365 days), medication adherence was assumed during the prescribed period. Monthly bisphosphonate exposure was treated as a time-varying variable, as treatment initiation was not standardized across patients. To minimize the influence of poor adherence, only individuals with an actual prescription adherence rate exceeding 80% were included. The adherence rate was calculated as:

$$\frac{\text{Total days of prescription}}{(\text{First day of BPP use–Last day of BPP use}+1)} \times 100\%.$$

Bone mineral density (BMD) was routinely evaluated on an annual basis after transplantation. Bisphosphonate therapy was initiated in cases of osteoporosis (defined as a T-score< -2.5) or according to individualized fracture risk determined by the physician. Baseline BMD was evaluated using dual-energy X-ray absorptiometry conducted within 1 year before or after transplantation. T-scores were calculated according to the standards of the third National Health and Nutrition Examination Survey, referencing data from young adult White females.[14]

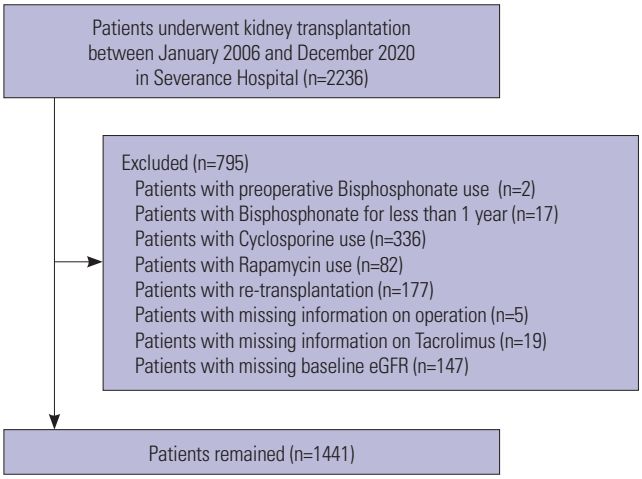

Fig. 1. Flowchart of the study populations. eGFR, estimated glomerular filtration rate.

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TTL

To calculate the TTL, all tacrolimus measurements obtained during outpatient visits throughout the post-transplantation study period were collected. To ensure accuracy and consistency, only values measured between 6:00 AM and 9:00 AM were included in the analysis. Individual TTLs were averaged at 3-month intervals and then converted into 1-month interval variables for statistical analysis. Blood samples were collected in ethylenediaminetetraacetic acid tubes and analyzed using the chemiluminescent microparticle immunoassay method (Dimension RxL Max; Reagent: Dimension TACR Flex TM Reagent Cartridge; Manufacturer: Siemens, Muich, Germany).

Immunosuppressive strategy

For induction therapy, basiliximab was administered on the day of surgery and again on postoperative day 4. Antithymocyte globulin was administered within 1 week of transplantation, primarily in patients with high immunologic risk. The majority of patients received a triple immunosuppressive regimen consisting of tacrolimus, antimetabolites, and methylprednisolone. Tacrolimus was initiated at a dose of 0.1 mg/kg/day from the day before surgery and was subsequently adjusted to maintain a target trough level of 3–8 ng/mL. The initial antimetabolites doses were as follows: mycophenolate mofetil, 1000–2000 mg/day; mycophenolate sodium, 720–1440 mg/day; azathioprine, 2 mg/kg/day; and mizoribine, 25–50 mg twice daily. Methylprednisolone was initiated at 500–1000 mg/day on the day of surgery and tapered to oral prednisolone at 5–10 mg/day within 1 month post-transplantation.

Outcome measurements

The co-primary outcomes were: 1) overall graft loss, defined as either patient death or initiation of kidney replacement therapy, and 2) an estimated glomerular filtration rate (eGFR) outcome, specifically an eGFR <30 mL/min/1.73 m^2, given that bisphosphonate therapy is generally not recommended below this threshold. Serum creatinine levels were measured using an enzymatic method traceable to isotope-dilution mass spectrometry, and eGFR was calculated with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[15] Fracture events were included as a secondary outcome. Incident fractures were identified using ICD-10 diagnostic codes corresponding to any fracture site, based on records from hospitalizations or outpatient visits (S02.x, S12.x, S22.x, S32.x, S42.x, S52.x, S62.x, S72.x, S82.x, and S92.x).

Statistical analysis

Baseline characteristics according to bisphosphonate use were compared using the Mann–Whitney test for non-parametric continuous variables and the chi-squared test for categorical variables. Baseline characteristics according to mean TTLs were compared using trend analysis with Cuzick's test for non-parametric continuous variables and the Cochran–Armitage test for categorical variables. As no continuous variable followed a normal distribution, all were reported as medians with interquartile ranges. Categorical variables were presented as numbers and percentages. Time-dependent Cox proportional hazards regression analysis was performed to evaluate the association between TTL and clinical outcomes according to bisphosphonate use. Survival time was defined as the time interval between the kidney transplantation and the occurrence of each co-primary outcome. Hazard ratios (HRs) were calculated using the cross-tabulation of TTLs and bisphosphonate use. Both TTLs and bisphosphonate use were treated as time-dependent variables with a time interval of 1 month. TTLs were stratified as above or below 4, 5, and 6 ng/mL. Multivariable Cox regression analyses were performed using the following confounding factors: recipient age, recipient sex, donor age, donor sex, recipient-donor relationship (deceased, living-related, or living-unrelated), HLA mismatch (0, 1–3, or 4–6), ABO mismatch (identical, compatible, or incompatible), history of desensitization, delayed graft function (DGF; defined as the requirement for hemodialysis within the first week after transplantation), acute rejection within 6 months post-transplant, diabetes history (none, pre-transplantation, or post-transplantation), cardiovascular disease, baseline eGFR, transplantation year, and bisphosphonate adherence. The proportional hazards assumption was verified using the scaled Schoenfeld residual method. Several sensitivity analyses were performed. First, the association between intrapatient variability (IPV) of TTLs and clinical outcomes, stratified by bisphosphonate use, was analyzed. IPV was calculated as the coefficient of variation using the following equation:

$$\text{Standard deviation}/X_{mean}\times 100.$$ [16]

To minimize early-phase variability, only TTLs measured between 6 and 18 months post-transplantation were included. Patients who experienced outcomes or were censored before 18 months were excluded. Second, to control for guarantee-time bias, a landmark analysis was conducted with a time point set at 3 months post-transplant. Third, the associations between minimum TTLs and clinical outcomes stratified by bisphosphonate use were analyzed to determine the effect of the lowest tacrolimus level. Fourth, patients with persistent hyperparathyroidism after transplantation (n=78) were excluded to minimize selection bias. Persistent hyperparathyroidism was assessed in 970 patients with available parathyroid hormone (PTH), albumin, and calcium levels between 6 months and 12 months post-transplant, and defined as corrected calcium >10.5 mg/dL and PTH >65 pg/mL. Fifth, a ≥50% decline in eGFR from baseline was adopted as an alternative endpoint. Sixth, in a subset of patients with available BMD data (n=966), the models were additionally adjusted for baseline femur BMD to account for potential confounding by underlying osteoporosis. Seventh, to evaluate the association between bisphosphonate use and frac-

ture prevention, post-transplant fracture events were analyzed as a secondary outcome. All statistical analyses were performed using the software Stata 18.0 (StataCorp, College Station, TX, USA), and p values<0.05 (two-tailed) were considered statistically significant.

Table 1. Baseline Characteristics of Patients according to Bisphosphonate Use

	Total (n=1441)	BPP user (n=262)	BPP non-user (n=1179)	*p*
Recipient demographics				
Age	48.0 (37.0–56.0)	50.0 (44.0–56.0)	47.0 (36.0–55.0)	<0.001
Sex, male	831 (57.7)	125 (47.7)	706 (59.9)	<0.001
DM history				
None	1084 (75.2)	212 (80.9)	872 (74.0)	0.002
Pre-transplantation	330 (22.9)	41 (15.6)	289 (24.5)	
Post-transplantation	27 (1.9)	9 (3.4)	18 (1.5)	
CVD history	121 (8.4)	22 (8.4)	99 (8.4)	0.999
Donor demographics				
Donor age	46.0 (35.0–53.0)	47.0 (36.0–55.0)	46.0 (35.0–53.0)	0.209
Donor sex, male	693 (48.1)	134 (51.1)	559 (47.4)	0.276
Donor type				0.265
Deceased	637 (44.2)	106 (40.5)	531 (45.0)	
Living related	401 (27.8)	83 (31.7)	318 (27.0)	
Living unrelated	403 (28.0)	73 (27.9)	330 (28.0)	
Transplant-related factors				
HLA mismatch				0.947
None	155 (10.8)	27 (10.3)	128 (10.9)	
1–3	780 (54.1)	141 (53.8)	639 (54.2)	
4–6	506 (35.1)	94 (35.9)	412 (34.9)	
ABO incompatibility				0.162
Identical	941 (65.3)	182 (69.5)	759 (64.4)	
Compatible	271 (18.8)	48 (18.3)	223 (18.9)	
Incompatible	229 (15.9)	32 (12.2)	197 (16.7)	
Immunosuppressant*				
Corticosteroid, mg	12.3 (10.0–12.9)	12.3 (11.0–13.0)	12.3 (9.8–12.8)	0.201
Mycophenolate, mg	903.6 (644.9–1000)	867.9 (625.6–1000)	906.3 (651.9–1000)	0.611
Desensitization	298 (20.7)	52 (19.8)	246 (20.9)	0.713
Delayed graft function	142 (9.9)	27 (10.3)	115 (9.8)	0.794
Acute rejection within 6 months	199 (13.8)	26 (9.9)	173 (14.7)	0.044
Baseline eGFR	66.1 (21.4)	66.4 (18.5)	66.1 (22.0)	0.826
Transplantation year				<0.001
2006–2010	328 (22.8)	98 (37.4)	230 (19.5)	
2011–2015	483 (33.5)	74 (28.2)	409 (34.7)	
2016–2020	630 (43.7)	90 (34.4)	540 (45.8)	
Bone parameters				
DEXA (T-score)†				
Lumbar	-0.9 (-1.9 to 0.1)	-1.8 (-2.6 to -0.9)	-0.7 (-1.6 to 0.3)	<0.001
Femur	-1.4 (-2.1 to -0.7)	-2.1 (-2.6 to -1.5)	-1.3 (-2.0 to -0.6)	<0.001
Hip	-0.9 (-1.7 to -0.1)	-1.6 (-2.3 to -1.0)	-0.7 (-1.5 to 0.0)	<0.001
Fracture history	28 (1.9)	8 (3.1)	20 (1.7)	0.150
Fracture event	106 (7.4)	33 (12.6)	73 (6.2)	<0.001

BPP, bisphosphonate; CVD, cardiovascular disease; DEXA, dual-energy X-ray absorptiometry; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen

Continuous variables are expressed as mean and standard deviation and frequency variables are expressed as absolute number and percentage.

*Median dose for each immunosuppressant was calculated for 1 year after surgery. For corticosteroids, the equivalent dose was calculated based on deflazacort. For mycophenolate, the equivalent dose was calculated based on mycophenolate mofetil; †Bone mineral density data were available for 966 patients.

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RESULTS

Study population and baseline characteristics

Of the 1441 KTRs, 262 (18.2%) were bisphosphonate users and 1179 (81.8%) were non-users. During a median follow-up of 6.1 (3.4–9.7) years, bisphosphonate users were treated for a median duration of 3.3 (1.8–7.3) years, covering 59.2% (33.6–78.9) of the total follow-up period. The mean prescription adherence rate was 97.4±5.9%. Bisphosphonates were initiated at a median of 1.3 (0.8–3.8) years post-transplant and discontinued at 6.3 (3.0–10.0) years. Baseline characteristics by bisphosphonate use are summarized in Table 1. The median recipient age was 48.0 (37.0–56.0) years, and 57.7% were male. Compared to non-users, bisphosphonate users were older, had a lower prevalence of pre-transplant diabetes, and underwent transplant earlier. They also exhibited lower BMD and a higher incidence of prior fractures. No significant differences were observed in HLA or ABO incompatibility status, desensitization, DGF, corticosteroid and mycophenolate dosages, or baseline kidney function (defined as the latest value measured between 3 and 6 months post-transplant). TTLs were consistently lower in bisphosphonate users than in non-users (Fig. 2).

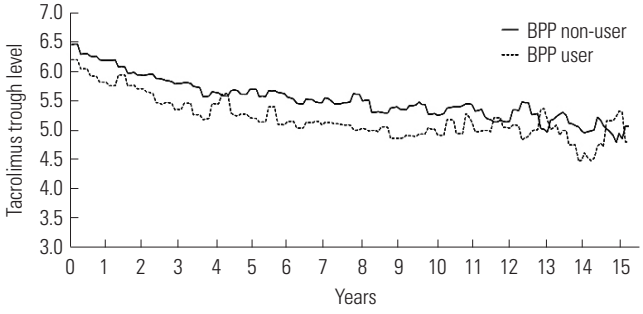

Fig. 2. Tacrolimus trough levels according to bisphosphonate use. BPP, bisphosphonate.

Association between bisphosphonate use and clinical outcomes

Bisphosphonate use was significantly associated with improved long-term graft survival and better preservation of kidney function. During a total follow-up of 9855.3 person-years [median, 6.1 (3.4–9.7) years], overall graft loss occurred in 157 (10.9%) patients {incidence rate, 15.9 per 1000 person-years [95% confidence interval (CI) 13.6–18.6]} (Table 2). The proportion of graft loss was 3.8% among bisphosphonate users and 12.5% among non-users. Cox regression analysis revealed a significant association between bisphosphonate use and a lower risk of overall graft loss (HR, 0.23; 95% CI, 0.12–0.43) compared with non-use. This association persisted even after adjusting for confounding factors [adjusted HR (aHR), 0.23; 95% CI, 0.12–0.44].

Regarding kidney function, over 9110.1 person-years of follow-up, 253 (17.6%) patients experienced a decline in eGFR to <30 mL/min/1.73 m^2, with an incidence rate of 27.8 per 1000 person-years (95% CI, 24.6–31.4). The aHR of bisphosphonate use for the eGFR outcome was 0.43 (95% CI, 0.28–0.64). Although the incidence of acute rejection was lower among bisphosphonate users compared to non-users, the difference did not reach statistical significance (17.1% vs. 22.0%, p=0.06).

Stratified associations between TTLs and clinical outcomes according to bisphosphonate use

The associations between TTLs and clinical outcomes were evaluated by stratifying patients based on bisphosphonate use (Table 3). In a univariable Cox regression analysis, as expected, a TTL ≥6 ng/mL without bisphosphonate use was associated with a lower risk for overall graft loss (HR, 0.72; 95% CI, 0.50–0.99) compared to a TTL <6 ng/mL without bisphosphonate use. TTL ≥6 ng/mL with bisphosphonate use was associated with an even lower risk for overall graft loss (HR, 0.22; 95% CI, 0.07–0.71) compared to TTL <6 ng/mL without bisphospho-

Table 2. Clinical Outcomes according to Bisphosphonate Use

	Person-years	Events	Incidence rate	Overall graft loss			
				Unadjusted		Adjusted*	
				HR (95% CI)	p	HR (95% CI)	p
BPP non-use	7639.1	147 (12.5)	19.2 (16.4–22.6)	Reference		Reference	
BPP use	2216.2	10 (3.8)	4.5 (2.4–8.4)	0.23 (0.12–0.43)	<0.001	0.23 (0.12–0.44)	<0.001
Total	9855.3	157 (10.9)	15.9 (13.6–18.6)				
	Person-years	Events	Incidence rate	eGFR <30 mL/min/1.73 m^2			
				Unadjusted		Adjusted*	
				HR (95% CI)	p	HR (95% CI)	p
BPP non-use	7002.6	227 (19.3)	32.4 (28.5–36.9)	Reference		Reference	
BPP use	2107.5	26 (9.9)	12.3 (8.4–18.1)	0.39 (0.26–0.59)	<0.001	0.43 (0.28–0.64)	<0.001
Total	9110.1	253 (17.6)	27.8 (24.6–31.4)				

BPP, bisphosphonate; CI, confidence interval; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; HR, hazard ratio. Incidence rate is presented as per 1000 person-years.
*Adjusted for recipient age, recipient sex, donor age, donor sex, recipient-donor relation (deceased, living-related, or living-unrelated), HLA mismatch (0, 1–3, or 4–6), ABO mismatch (identical, compatible, or incompatible), DM history (none, pre-transplantation, or post-transplantation), baseline eGFR, and transplantation year.

Table 3. Cox Regression Analysis for Kidney Outcomes according to Mean TTLs and Bisphosphonate Use

	Overall graft loss				eGFR <30 mL/min/1.73 m²			
	Unadjusted		Adjusted		Unadjusted		Adjusted	
	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*
TTL <6 without BPP	Reference		Reference		Reference		Reference	
TTL ≥6 without BPP	0.72 (0.50–0.99)	0.050	0.65 (0.45–0.95)	0.026	1.01 (0.77–1.33)	0.924	1.01 (0.77–1.32)	0.955
TTL <6 with BPP	0.17 (0.08–0.37)	<0.001	0.20 (0.09–0.43)	<0.001	0.23 (0.13–0.41)	<0.001	0.29 (0.17–0.52)	<0.001
TTL ≥6 with BPP	0.22 (0.07–0.71)	0.011	0.25 (0.08–0.80)	0.019	0.21 (0.08–0.56)	0.002	0.27 (0.10–0.73)	0.010
TTL <5 without BPP	Reference		Reference		Reference		Reference	
TTL ≥5 without BPP	0.51 (0.37–0.71)	<0.001	0.48 (0.34–0.67)	<0.001	0.76 (0.59–0.98)	0.037	0.77 (0.59–1.00)	0.046
TTL <5 with BPP	0.11 (0.04–0.29)	<0.001	0.12 (0.04–0.34)	<0.001	0.22 (0.12–0.42)	<0.001	0.29 (0.15–0.54)	<0.001
TTL ≥5 with BPP	0.21 (0.09–0.48)	<0.001	0.23 (0.10–0.54)	0.001	0.16 (0.07–0.35)	<0.001	0.20 (0.09–0.46)	<0.001
TTL <4 without BPP	Reference		Reference		Reference		Reference	
TTL ≥4 without BPP	0.43 (0.31–0.60)	<0.001	0.41 (0.30–0.58)	<0.001	0.65 (0.50–0.87)	0.003	0.68 (0.51–0.90)	0.007
TTL <4 with BPP	0.14 (0.05–0.39)	<0.001	0.16 (0.06–0.45)	<0.001	0.28 (0.14–0.56)	<0.001	0.38 (0.19–0.77)	0.007
TTL ≥4 with BPP	0.10 (0.04–0.24)	<0.001	0.12 (0.05–0.28)	<0.001	0.11 (0.05–0.23)	<0.001	0.15 (0.07–0.31)	<0.001

BPP, bisphosphonate; CI, confidence interval; CVD, cardiovascular disease; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; HR, hazard ratio; TTL, tacrolimus trough level.
Adjusted for recipient age, recipient sex, donor age, donor sex, recipient-donor relation (deceased, living-related, or living-unrelated), HLA mismatch (0, 1–3, or 4–6), ABO mismatch (identical, compatible, or incompatible), history of desensitization, delayed graft function, acute rejection within 6 months post-transplant, DM history (none, pre-transplantation, or post-transplantation), CVD history, baseline eGFR, transplantation year, and bisphosphonate adherence.

Table 4. Cox Regression Analysis for Kidney Outcomes according to IPV of Tacrolimus Trough Levels and Bisphosphonate Use

	Overall graft loss				eGFR <30 mL/min/1.73 m²			
	Unadjusted		Adjusted		Unadjusted		Adjusted	
	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*
High IPV without BPP	Reference		Reference		Reference		Reference	
Low IPV without BPP	0.66 (0.45–0.97)	0.036	0.73 (0.50–1.09)	0.123	0.63 (0.47–0.84)	0.001	0.64 (0.48–0.86)	0.003
High IPV with BPP	0.24 (0.10–0.59)	0.002	0.26 (0.10–0.64)	0.004	0.35 (0.20–0.62)	<0.001	0.36 (0.20–0.64)	0.001
Low IPV with BPP	0.24 (0.10–0.60)	0.002	0.23 (0.09–0.59)	0.002	0.32 (0.18–0.57)	<0.001	0.35 (0.19–0.63)	0.001

BPP, bisphosphonate; CI, confidence interval; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; HR, hazard ratio; IPV, intrapatient variability.
Adjusted for recipient age, recipient sex, donor age, donor sex, recipient-donor relation (deceased, living-related, or living-unrelated), HLA mismatch (0, 1–3, or 4–6), ABO mismatch (identical, compatible, or incompatible), history of desensitization, delayed graft function, acute rejection within 6 months post-transplant, DM history (none, pre-transplantation, or post-transplantation), CVD history, baseline eGFR, transplantation year, and bisphosphonate adherence.

nate use. Interestingly, when bisphosphonate was used, a TTL <6 ng/mL also showed a lower risk of overall graft loss (HR, 0.17; 95% CI, 0.08–0.37) than a TTL <6 ng/mL without bisphosphonate use. These associations persisted even after adjusting for confounding factors (TTL ≥6 ng/mL without bisphosphonate use, aHR, 0.65; 95% CI, 0.45–0.95; TTL <6 ng/mL with bisphosphonate use, aHR, 0.20; 95% CI, 0.09–0.43; TTL ≥6 ng/mL with bisphosphonate use, aHR, 0.25; 95% CI, 0.08–0.80). The associations remained consistent when a TTL of 4 or 5 ng/mL was used as a reference. Similar associations were observed for the outcome of eGFR <30 mL/min/1.73 m².

Sensitivity analyses

Various sensitivity analyses were conducted to verify our main findings. Firstly, when IPV values [median: 14.7 (10.0–21.6)%] were used for the analysis, a low IPV without bisphosphonate use showed a tendency toward a lower risk for overall graft loss (aHR, 0.73; 95% CI, 0.50–1.09) compared to a high IPV without bisphosphonate use, although this did not reach statistical significance. When bisphosphonate was used, a low IPV was associated with a significantly reduced risk for overall graft loss (aHR, 0.23; 95% CI, 0.09–0.59). Interestingly, even a high IPV was associated with a lower risk for overall graft loss when bisphosphonate was concurrently used (aHR, 0.26; 95% CI, 0.10–0.64) (Table 4). Secondly, a landmark analysis was conducted with a 3-month time lag, and the results were consistent with the main analysis for both overall graft loss and the eGFR outcome (Supplementary Table 1, only online). Thirdly, when minimum TTLs were used for the analysis, similar results were observed (Supplementary Table 2, only online). Fourthly, even after excluding patients with persistent hyperparathyroidism, the results remained consistent (Supplementary Table 3, only online). Fifth, when an alternative endpoint of ≥50% decline in eGFR was used, similar results were observed (Supplementary

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Table 4, only online). The favorable association was observed only among patients receiving bisphosphonates, and this association was primarily limited to those with TTLs <6 ng/mL. Sixth, after additional adjustment for baseline femur BMD to account for potential confounding by underlying osteoporosis, similar results were observed (Supplementary Table 5, only online). Lastly, in a univariable Cox regression analysis for fracture outcome, bisphosphonate use was not associated with the fracture outcome (HR, 0.88; 95% CI, 0.56–1.38). However, when recipient age, history of diabetes and fracture, and baseline femur T-score were adjusted, bisphosphonate use was associated with a significantly lower risk of fracture events (aHR, 0.54; 95% CI, 0.28–0.99) (Supplementary Table 6, only online).

DISCUSSION

A prospective study examining the development of chronic allograft nephropathy identified that extended exposure to CNIs was correlated with the progression of ischemic glomerulonephritis and arteriolar hyalinosis.[17] Accordingly, strategies to reduce CNI exposure have been explored in many randomized controlled trials. The Symphony trial demonstrated for the first time that a low-dose tacrolimus regimen was superior to a low-dose cyclosporine or sirolimus regimen, or a standard-dose tacrolimus regimen in kidney function, allograft survival, and acute rejection.[4] In this trial, the target trough level for the low-dose tacrolimus was set between 3–7 ng/mL, with an actual mean trough level of 6.6 ng/mL achieved during the first post-transplant year. Conversely, studies that set the TTL to less than 6 ng/mL during the first post-surgery year found a significantly heightened risk for graft loss and acute rejection.[18] Subsequent studies have hence aimed to discover optimal regimens that would allow a reduction in the dosage of tacrolimus by employing combinations of different immunosuppressive agents. Particularly, sirolimus and everolimus, inhibitors of the mammalian target of rapamycin (mTOR), have been found to synergistically enhance the immunosuppressive action of CNIs. Several studies have thus explored whether substituting mycophenolate mofetil with an mTOR inhibitor could allow a reduction in the required dosage of tacrolimus.[19,20] However, it was found that combinations of low-dose tacrolimus and an mTOR inhibitor resulted in a higher incidence of efficacy failure owing to intolerable adverse events, although the outcomes of kidney function and graft survival were comparable to those achieved with mycophenolate mofetil therapy.[19,20] Recently, our team found a correlation between the use of bisphosphonate and favorable long-term graft outcomes in KTRs.[8] Furthermore, other research groups have discovered associations between the use of ibandronate and both favorable long-term graft outcomes and decreased acute rejection in KTRs.[9,10] As experimental studies have shown that bisphosphonates have immunomodulatory effects on both innate and adaptive immunity, we suggest that bisphosphonates could serve as effective adjunctive immunosuppressant agents in KTRs.

Our investigation next turned to the possible tacrolimus-sparing effects of bisphosphonates. For this, we focused our analysis specifically on patients who had undergone kidney transplantation and were receiving tacrolimus-based immunosuppressive therapy. As hypothesized, bisphosphonate use corresponded with a diminished risk of fracture. Beyond this primary therapeutic application, bisphosphonate use was also linked with favorable graft outcomes even among patients with lower TTLs. Bisphosphonate users displayed significantly lower TTLs when compared with non-users. We hypothesize that this decrease in tacrolimus exposure may have contributed to the observed favorable graft outcomes by reducing CNI toxicity. Interestingly, rejection events were also less frequent among bisphosphonate users. This occurred despite no significant differences in the dosages of mycophenolate and corticosteroids, suggesting a potential synergistic effect with CNIs in bisphosphonate users. We also observed that bisphosphonate use was linked with positive outcomes even in patients experiencing high IPV of tacrolimus. As high IPV of tacrolimus often corresponds with adverse transplantation outcomes due to unintended under-immunosuppression or over-exposure to tacrolimus,[16] it is plausible that bisphosphonate use could provide a broader therapeutic window for tacrolimus.

Regarding safety, bisphosphonates are not recommended for patients with severe renal impairment (creatinine clearance <30 or 35 mL/min/1.73 m^2) due to their potential nephrotoxicity.[21] However, even when the outcome was set as eGFR <30 mL/min/1.73 m^2, bisphosphonate use remained associated with favorable outcomes even at lower TTLs. This particular analysis helped to minimize potential indication bias that might result from discontinuation of bisphosphonate in advanced CKD. The safety profile of bisphosphonates, established over more than 30 years of use, is a significant advantage, especially given the safety concerns surrounding many immunosuppressants despite their potent immunosuppressive effects. Although more evidence is needed regarding the safety and efficacy of bisphosphonates in patients with advanced CKD, our findings show a slower rate of kidney function decline in bisphosphonate users, indicative of their renal safety in KTRs. This could be attributed to the fact that bisphosphonates were administered at doses typically used for osteoporosis treatment in this study. Notably, bisphosphonate-related acute kidney injury has been reported only in cases involving frequent and rapid infusions in cancer patients,[22-24] while no significant differences in renal impairment were observed in studies involving osteoporosis patients.[25,26]

The immunomodulatory effects of bisphosphonates have been explored in several studies. Early investigations reported that bisphosphonate use led to in vivo expansion of gamma-delta (γδ) T cells in certain cancer patients.[27-30] This occurs because bisphosphonates and their metabolites activate γδ T cells

by accumulating mevalonate pathway metabolites in cells.[31,32] However, recent studies in children, postmenopausal women, and the elderly found that bisphosphonate treatment eventually reduces circulating γδ T cells.[33-36] This is attributed to activation-induced cell death and neutrophil-mediated T cell inhibition.[37,38] Bisphosphonates also hinder T cell proliferation by impairing cytokine production from antigen-presenting cells.[39] Pretreatment of monocytes with alendronate showed dose-dependent inhibition of interleukin-1β, interleukin-6, and tumor necrosis factor-α production. These findings suggest that bisphosphonates exert T cell–mediated immunomodulatory effects that may vary by patient population. Given that this T cell-mediated immunomodulatory effect of bisphosphonates partially mirrors the mechanism of T cell inhibition by tacrolimus,[40] the concurrent use of bisphosphonates with tacrolimus might amplify the immunomodulatory effect. Thus, a controlled trial is needed to confirm bisphosphonates' tacrolimus-sparing effect, and further experimental studies are warranted to elucidate underlying mechanisms.

This study has several limitations. First, its retrospective design limits the ability to establish a causal relationship. Nevertheless, our models adjusted for multiple confounders. The co-primary outcome analysis using eGFR <30 mL/min/1.73 m^2, along with sensitivity analyses based on minimum and IPV of tacrolimus levels, yielded consistent findings. Likewise, analyses excluding patients with persistent hyperparathyroidism, which is known to be associated with graft loss, confirmed similar findings. Additionally, a landmark analysis further reduced guarantee-time bias that can occur in retrospective pharmaceutical research. Secondly, we did not follow a specific protocol for measuring tacrolimus levels. However, we selected only the measurements taken between 6 AM and 9 AM to represent the TTL and accounted for dynamic changes in tacrolimus by treating them as time-varying variables at 3-month intervals. Thirdly, we were unable to confirm actual bisphosphonate intake as usage was assessed based on prescription records. Accurate data regarding the dosage and duration of bisphosphonate intake are essential to validate our study's results. Fourthly, classification based on bisphosphonate use could introduce selection biases such as healthy user bias. However, it is important to emphasize that our study was conducted at a single center, resulting in a relatively homogeneous study population, which helps to mitigate this concern. Finally, due to the non-randomized nature of bisphosphonate use, there is potential for selection bias, although adjustment for femur neck BMD was performed to mitigate this concern.

In conclusion, our findings indicate that bisphosphonate users experienced more favorable graft outcomes while requiring a lower dose of tacrolimus. This suggests the potential of a tacrolimus-sparing effect of bisphosphonates in KTRs. Given the well-established safety profile of bisphosphonates, their utilization could be a more immediate solution to reducing CNI toxicity compared to the development of new immunosuppressant drugs. To validate these findings, further controlled trials are needed to verify the immunosuppressive and tacrolimus-sparing effects of bisphosphonates in KTRs.

DATA AVAILABILITY STATEMENT

Study protocol: The study protocol is available upon request. Statistical codes: The statistical codes used for analyses are available from Dr. Kim (e-mail, docbsk@yuhs.ac). Data set: Severance Hospital transplantation data are not publicly available since the ownership belongs to the Yonsei University Health System. However, the data may be provided upon reasonable request.

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