Transplant Infectious Disease

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Association of Posttransplant Circulating 25-Hydroxyvitamin D and Late-Onset Infections Among Kidney Transplant Recipients: The Wisconsin Allograft Recipient Database (WisARD)

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ABSTRACT

Introduction: Late-onset infection occurring more than 6 months after transplantation is a major threat to the long-term survival of kidney transplant recipients (KTRs). Accumulating evidence indicates a potential role for vitamin D in host resistance to infections. While vitamin D inadequacy is common among KTRs, the association of posttransplant circulating 25-hydroxyvitamin D [25(OH)D] and late-onset infection remains uncertain.

Methods: We analyzed data from adult kidney-only transplant recipients at our center from 2005 to 2020 who had at least one valid posttransplant circulating 25(OH)D measurement from 5 to 13 months posttransplant. Survival analyses were conducted using marginal proportional rates models with late-onset infection within 1 year following the 25(OH)D measurement as the event of interest. Additional analyses used time-varying 25(OH)D measurements.

Results: Of 2207 KTRs included, 642 recipients had a total of 1448 late-onset infection episodes. Each 5 ng/mL lower serum 25(OH)D was associated with a 5% higher risk of late-onset infection (adjusted rate ratio [aRR] = 1.05; 95% confidence interval [CI]: 1.03, 1.07; $p < 0.01$). Vitamin D deficiency (≤ 20 ng/mL) was associated with a 1.22-fold higher incidence of late-onset infection (aRR = 1.22; 95% CI: 1.03–1.43; $p = 0.02$) compared with vitamin D sufficiency (≥30 ng/mL). The association was strongest for urinary tract infection among male recipients (aRR = 2.20; 95% CI: 1.57–3.08; $p < 0.01$).

Conclusion: Vitamin D deficiency is significantly associated with a higher incidence of late-onset infection among KTRs, especially urinary tract infections in male recipients. Further research, including clinical trials, is needed to determine the causal relationship.

1 | Introduction

Infection remains a leading cause of hospitalization and mortality among kidney transplant recipients (KTRs) due to chronic immunosuppression [1]. Posttransplant infection is heterogeneous and usually categorized according to the time of disease onset [2]. Most infections happening in the first month posttransplant are surgery-related nosocomial infections [3]. Opportunistic infections typically occur in the first 6 months posttransplant under a peak exposure to immunosuppression [2]. Late-onset infections after 6 months are mostly community-acquired and commonly include pneumonia and urinary tract infection (UTI) [4].

Prevention of late-onset infection is crucial in the long-term management of KTRs. Late-onset infection is often hard to recognize among KTRs with mild signs and symptoms compared to patients with normal immune function. Complicated late-onset infection often results in hospitalization with excess financial burden. Some late-onset infections, especially recurrent UTIs, are detrimental to allograft function and patient survival [5–7]. Vaccination, prophylaxis, and preemptive therapy have been widely applied in late-onset infection prevention [4]. Lifelong prophylaxis remains in discussion considering the potential risk of drug resistance and the challenge of immunosuppression management. There remains, however, a critical need for additional prevention strategies.

Vitamin D inadequacy is prevalent among KTRs as a result of reduced sun exposure and increased catabolism induced by immunosuppressive drugs [8–12]. There is increasing recognition of the immunoregulatory role of vitamin D and growing interest in its potential for infection prevention. 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], the biologically active form of vitamin D, is a potent immunomodulator [13]. Enzymes involved in vitamin D metabolism and related receptors are widely expressed by various immune cells [14–16]. Experimental studies have shown multiple properties of 1,25$(OH)_2$D in antibacterial and antiviral immunity [17–20].

Numerous observational studies and clinical trials have been conducted to investigate the antimicrobial effect of vitamin D in susceptible populations, but results are inconsistent [21–23]. Evidence is limited among KTRs, a large and growing immunocompromised population with vitamin D metabolism interrupted by immunosuppressants. We investigated the association between posttransplant circulating 25(OH)D and the incidence of late-onset infection among a large cohort of KTRs.

2 | Methods

2.1 | Study Population and Data Sources

We used data from the Wisconsin Allograft Recipient Database (WisARD). WisARD longitudinally collects information on clinic visits, laboratory tests, morbidities, and allograft outcomes of all solid-organ transplants performed at the University of Wisconsin (UW) [24]. We included all adult (≥ 18 years old) kidney-only transplant recipients at UW from January 1, 2005 to December 31, 2020, who had at least one serum 25(OH)D measurement during the baseline window (i.e., 5–13 months posttransplant) and had a functioning allograft at the time of the first 25(OH)D measurement (eGFR ≥ 15 mL/min/1.73m^2). The baseline window was defined as 5–13 months after transplantation in light of the substantial fluctuation of serum 25(OH)D levels in the first few months. All subsequent 25(OH)D measurements also were collected for complementary time-varying analyses. Recipients without concurrent baseline measurements (i.e., within ±14 days of vitamin D measurement) of serum albumin, urine protein–creatinine ratio (UPCR), or estimated glomerular filtration rate (eGFR) were excluded. Only the most recent transplant for each recipient within the study period was included to ensure independence in analysis. This study was approved by the University of Wisconsin Health Sciences Institutional Review Board.

2.2 | Posttransplant Circulating 25(OH)D

Recipients at our center routinely received 2000 IU/day of cholecalciferol starting at the time of transplantation. Measurement of serum 25(OH)D levels was scheduled at 6 months posttransplant, then yearly as needed. All posttransplant serum 25(OH)D measurements of eligible recipients were collected from the University of Wisconsin Hospital and Clinics Clinical Laboratory and other qualified laboratories where liquid chromatography tandem mass spectrometry (LC-MS/MS) was applied. Levels of serum 25(OH)D lower than the detectable threshold (i.e., 5 ng/mL) were regarded as missing. Posttransplant vitamin D status was defined as follows: vitamin D deficiency: ≤ 20 ng/mL (50 nmol/L); vitamin D insufficiency: 21–29 ng/mL (52.5–72.5 nmol/L); vitamin D sufficiency: ≥ 30 ng/mL (75 nmol/L) as suggested by the Endocrine Society [25]. Measurement time of posttransplant serum 25(OH)D was counted as months posttransplant. Season of serum 25(OH)D measurement was categorized into winter (December–February), spring (March–May), summer (June–August), and fall (September–November).

2.3 | Other Measurements

Demographics and clinical characteristics of eligible recipients at transplantation were collected, including age, sex, race, body mass index (BMI), cause of end-stage kidney disease (ESKD), donor status, previous kidney transplant, human leukocyte antigens (HLA) mismatch, and calendar year of transplantation. Other clinical characteristics following transplantation were collected as well, including the occurrence of delayed graft function (defined as the need for dialysis within the first week following transplantation), induction and maintenance immunosuppression, and a history of infections and acute rejection before the first 25(OH)D measurement in the baseline window. In addition, concurrent baseline serum albumin, UPCR, and serum creatinine levels were collected as potential confounders. GFR was estimated from serum creatinine using the race-neutral CKD-EPI creatinine equation (2021) [26].

2.4 | Late-Onset Infection

Eligible recipients were followed for 1 year from the time of each serum 25(OH)D measurement, allograft failure, death, or the

TABLE 1 | Baseline characteristics of eligible kidney transplant recipients

Characteristics	Serum 25(OH)D[a]				
	Total	≤20 ng/mL	21–29 ng/mL	≥30 ng/mL	
Number of KTRs	***N* = 2207**	***N* = 231**	***N* = 507**	***N* = 1469**	***p* value**
Age at transplant (yrs)	53 (13)	49 (14)	52 (13)	54 (12)	< 0.01
Male (%)	1377 (62)	132 (57)	316 (62)	929 (63)	0.21
Race (%)					< 0.01
White	1726 (78)	165 (71)	372 (73)	1189 (81)	
Non-White[b]	481 (22)	66 (29)	135 (27)	280 (19)	
BMI at transplant, kg/m^2	28 (5)	28 (6)	29 (5)	28 (5)	< 0.01
Cause of ESKD (%)					< 0.01
Diabetes	542 (25)	75 (32)	162 (32)	305 (21)	
Hypertension	289 (13)	32 (14)	60 (12)	197 (13)	
Glomerulonephritis	565 (26)	55 (24)	115 (23)	395 (27)	
PKD	319 (14)	18 (8)	45 (9)	256 (17)	
Other	492 (22)	51 (22)	125 (24)	316 (22)	
Living donor (%)	841 (38)	61 (26)	160 (32)	620 (42)	< 0.01
Prior kidney transplant (%)	452 (20)	58 (25)	125 (25)	269 (18)	< 0.01
HLA antigen mismatch (%)					0.78
0–2	404 (18)	45 (19)	98 (19)	261 (18)	
3–4	957 (44)	103 (45)	210 (41)	644 (44)	
5–6	846 (38)	83 (36)	199 (40)	564 (38)	
Delayed allograft function (%)	434 (20)	68 (29)	126 (25)	240 (16)	< 0.01
Induction immunosuppression (%)					< 0.01
Basiliximab	1257 (57)	110 (48)	300 (59)	847 (58)	
Alemtuzumab	207 (9)	31 (13)	36 (7)	140 (10)	
Antithymocyte globulin	708 (32)	86 (37)	169 (33)	453 (31)	
Other	35 (2)	4 (2)	29 (1)	35 (1)	
Maintenance tacrolimus (%)	2130 (97)	222 (96)	490 (97)	1418 (97)	0.03
History of infection before baseline (%)[c]	1066 (48)	118 (51)	277 (55)	671 (46)	< 0.01
Acute rejection before baseline (%)[c]	260 (12)	41 (18)	61 (12)	158 (11)	< 0.01
Transplant year (%)					< 0.01
2005–2009	395 (18)	29 (13)	89 (18)	277 (19)	
2010–2014	912 (41)	95 (41)	237 (47)	580 (39)	
2015–2020	900 (41)	107 (46)	181 (35)	612 (42)	
Serum 25(OH)D measurement time, months after transplant[d]	7.7 (6.5, 9.6)	8.0 (6.5, 9.8)	7.7 (6.5, 9.4)	7.7 (6.5, 9.7)	0.39
Season of 25(OH)D measurement (%)					< 0.01
Winter	582 (26)	76 (33)	148 (29)	358 (24)	
Spring	588 (27)	69 (30)	131 (26)	388 (27)	
Summer	520 (24)	34 (15)	117 (23)	369 (25)	
Fall	517 (23)	52 (22)	111 (22)	354 (24)	
Serum albumin (g/dL)	3.8 (3.6, 4.0)	3.7 (3.5, 4.1)	3.8 (3.5, 4.0)	3.8 (3.6, 4.1)	< 0.01
UPCR (mg/mg)	0.19 (0.12, 0.31)	0.24 (0.14, 0.50)	0.20 (0.12, 0.34)	0.18 (0.11, 0.29)	< 0.01

(Continues)

TABLE 1 | (Continued)

Characteristics	Serum 25(OH)D[a]				
	Total	≤20 ng/mL	21–29 ng/mL	≥30 ng/mL	
Number of KTRs	***N* = 2207**	***N* = 231**	***N* = 507**	***N* = 1469**	***p* value**
eGFR (%)[e]					0.08
≥ 60 mL/min/1.73m^2	878 (40)	112 (49)	211 (42)	555 (38)	
45–59 mL/min/1.73m^2	726 (33)	68 (29)	163 (32)	495 (34)	
30–44 mL/min/1.73m^2	485 (22)	42 (18)	109 (21)	334 (22)	
< 30 mL/min/1.73m^2	118 (5)	9 (4)	24 (5)	85 (6)	

Note: Continuous variables were presented as mean (standard deviation) or median (interquartile range). Categorical variables were presented as count (percentage).

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; eGFR, estimated glomerular filtration rate; ESKD, end stage kidney disease; HLA, human leukocyte antigens; KTR, kidney transplant recipients; PKD, polycystic kidney disease; UPCR, urine protein–creatinine ratio.

[a]The earliest serum 25(OH)D measurement during 5–13 months after transplantation.

[b]Included American Indian, Alaska Natives, Asian, and Pacific Islander.

[c]History of posttransplant infection before baseline serum 25(OH)D measurement.

[d]Measurement time of serum 25(OH)D was counted as months after transplantation and presented as median (interquartile range).

[e]The concurrent estimated glomerular filtration rate within 1 week of serum uric acid measurement. GFR was estimated using race-neutral CKD-EPI Creatinine Equation (2021).

end of the study (i.e., December 31, 2022), whichever came first. Incident late-onset infection episodes were identified via relevant diagnoses in the medical records. Clinical signs and symptoms of local or systemic infection were required for diagnosis. CMV infections included symptomatic episodes with corresponding lab evidence of viral replication. BK polyoma virus (BKPyV) infections included biopsy-proven or presumptive BKPyV-associated nephropathy. Waterborne, vector-borne, and parasitic infections were excluded. Surgical site infection and urinary tract candidiasis were excluded due to their close correlation with surgical procedures and indwelling urinary catheters during hospitalization. All other infection episodes of each recipient were collected. Late-onset infections were categorized into three strata according to their pathogens: bacterial, viral, and fungal infections. Antiviral prophylaxis, either with acyclovir or valganciclovir, was given for 3–6 months after transplantation to prevent CMV infection. Antibacterial prophylaxis with trimethoprim–sulfamethoxazole was given for 1 year posttransplant during the study period. Antifungal prophylaxis (topical nystatin) was prescribed for 1 month following a rejection episode. All antimicrobials were recycled after the treatment of rejection.

2.5 | Statistical Analysis

Baseline characteristics were compared across vitamin D categories with chi-square tests or logistic regression, as appropriate. The incidence rate of overall late-onset infection was calculated based on the first 25(OH)D measurement and multiple potential subsequent episodes per recipient. We also calculated the incidence rates for bacterial, viral, and fungal infections separately, again including multiple potential episodes. Cumulative infection-free survival was calculated and depicted via the Kaplan–Meier method. Marginal proportional rates models were fitted to account for the potential for multiple infection episodes for each recipient. Our primary analysis used the first 25(OH)D measurement during the baseline window as the exposure of interest. Sensitivity analyses were performed using all 25(OH)D measurements fitted as a time-varying variable.

Serum 25(OH)D level was modeled as a continuous variable and categorical variable as defined above. Other baseline characteristics mentioned above were included in multivariate models as potential confounders. The continuous association between 25(OH)D and outcomes also was fitted using a natural cubic spline with knots at 10, 50, and 90 percentiles to capture potential nonlinearity. Separate analyses were conducted for specific infections. Pretransplant CMV serology was further adjusted for in models of viral infection. Stratified analyses were performed by recipient age at transplantation and recipient sex. All analyses were performed using Stata Statistical Software Release 17.0 (www.stata.com).

3 | Results

A total of 4260 adult patients received a kidney-only transplant at our center from January 1, 2005 to December 31, 2020. A total of 2207 recipients who maintained a functioning allograft at the time of their first serum 25(OH)D measurement during the baseline window (i.e., 5–13 months posttransplant) were included in this study (Figure S1). There were no significant differences in baseline characteristics between included and excluded recipients, with the exception that more KTRs who received transplantation after 2010 were included. Eligible recipients were followed for a median of 6.6 years (interquartile range [IQR]: 3.9, 9.6) after transplantation. Most recipients had their first serum 25(OH)D measurement from 6 to 10 months posttransplant (Table 1). Approximately 23% of recipients had vitamin D insufficiency (21–29 ng/mL), and 10% had vitamin D deficiency (≤ 20 ng/mL) based on the first 25(OH)D measurement during the baseline window. Those with lower serum 25(OH)D levels were younger, more likely to be non-White, had higher BMI at transplant, and were more likely to have diabetes as the

cause of ESKD. Those with lower serum 25(OH)D levels also were more likely to have received a graft from a deceased donor, had a previous kidney transplant, and experienced delayed graft function. Antithymocyte globulin usage, history of infections, and history of acute rejections within 5 months posttransplant also were associated with a lower level of serum 25(OH)D. The prevalence of vitamin D inadequacy decreased over time. Serum 25(OH)D level was lower in winter and higher in summer as expected due to the reduced sunlight exposure in winter. We also found serum 25(OH)D level positively correlated with serum albumin and negatively correlated with UPCR.

3.1 | Overall Infection

In the 2207 KTRs included, a total of 1448 infection episodes occurred in 642 recipients within 1 year of the first 25(OH)D measurement. UTI was the most common type of infection (N = 612), followed by viremia (N = 263) and pneumonia (N = 100; Table S1). Approximately 50% of infections were bacterial infections, with the most prevalent bacterial pathogen identified being *Escherichia coli*, followed by *Klebsiella pneumoniae* and coagulase-negative *staphylococci*. Viral infection accounted for 28% of infections (N = 399). The majority were cytomegalovirus and BKPyV. Fungal infection, accounted for 5% of infections (N = 67), and 43% of these were *Candida* infections.

The incidence rate of overall infection was 67 episodes per 100 person-years. Recipients with vitamin D deficiency had the highest incidence of infection (Table 2) and the lowest infection-free survival rate (Figure 1). Each 5 ng/mL lower 25(OH)D level was associated with a 5% higher incidence of overall infection (adjusted rate ratio [aRR] = 1.05; 95% confidence interval [CI]: 1.01, 1.08; $p < 0.01$) after adjusting for potential confounders in Table 1. Posttransplant vitamin D deficiency was associated with a 1.19-fold higher incidence of overall infection (aRR = 1.19; 95% CI: 1.00, 1.52; $p = 0.05$) compared with vitamin D sufficiency (Table 2). The relationship was approximately linear across the range of 25(OH)D levels (Figure 2a).

3.2 | Bacterial Infection

A total of 721 bacterial infection episodes among 348 recipients were identified, with a median of two episodes (IQR: 1, 3) for each recipient. The estimated incidence of bacterial infection was 33 episodes per 100 person-years. Recipients with deficient vitamin D had a 1.7-fold higher incidence of bacterial infection than those with sufficient vitamin D (Table 2). Each 5 ng/mL lower posttransplant serum 25(OH)D level was significantly associated with a 5% higher incidence of bacterial infection (aRR = 1.05; 95% CI: 1.01, 1.10; $p = 0.04$) after adjusting for potential confounders (Table 2). No threshold for non-linearity was detected (Figure 2b).

UTI accounted for about 85% of bacterial infections identified. A total of 612 UTI episodes among 284 recipients were identified, with an incidence rate of 28 episodes per 100 person-years. There were at most nine episodes identified for each recipient, with a median of two episodes (IQR: 1, 3). The incidence among recipients with vitamin D deficiency was almost twice that among recipients with vitamin D sufficiency (Table 2). Each 5 ng/mL lower 25(OH)D was associated with a 7% higher incidence of UTI (aRR = 1.07; 95% CI: 1.02, 1.13; $p < 0.01$) after adjustment. Vitamin D deficiency was significantly associated with a 59% higher incidence of UTI (aRR = 1.59; 95% CI: 1.15–2.20; $p < 0.01$; Table 2). A linear association was observed without a specific threshold (Figure 2c).

3.3 | Viral Infection

A total of 399 viral infection episodes among 276 recipients were identified within 1 year of the first 25(OH)D measurement. The incidence was 1.4-fold higher among recipients with vitamin D deficiency compared with those with vitamin D sufficiency (Table 2). Each 5 ng/mL lower posttransplant circulating 25(OH)D level was associated with a 6% higher incidence of viral infection (aRR = 1.06; 95% CI: 1.01, 1.12; $p = 0.02$) in a univariate model. However, this association was not statistically significant after adjustment (Table 2). No significant threshold for nonlinearity was detected (Figure 2d).

3.4 | Fungal Infection

A total of 67 fungal infection episodes among 53 recipients were identified within 1 year of the first 25(OH)D measurement. The incidence was similar across vitamin D categories. No significant association between posttransplant circulating 25(OH)D and fungal infection was detected (Table 2). No significant threshold for nonlinearity was detected (Figure 2e).

3.5 | Stratified Analyses

The association between 25(OH)D levels and infection did not differ significantly by recipient age. Each 5 ng/mL lower level was associated with a 7% higher incidence in older recipients (≥ 65 years old) and a 4% higher incidence in younger recipients (< 65 years old; p interaction = 0.29). Similarly, no interaction was detected for bacterial, viral, or fungal infection (Table S2).

A total of 245 UTI episodes occurred in 107 male recipients and a total of 367 UTI episodes occurred in 177 female recipients. The incidence was 2.5-fold higher among female recipients versus male recipients (45 episodes per 100 person-years in women versus 18 episodes per 100 person-years in men). The association of 25(OH)D with UTI, however, was significantly stronger in male compared to female recipients (Table S3, p interaction = 0.02). Each 5 ng/mL lower posttransplant circulating 25(OH)D level was associated with an 11% higher incidence of UTI (aRR = 1.11; 95% CI: 1.04, 1.18; $p < 0.01$) among male recipients and a 5% higher incidence (aRR = 1.05; 95% CI: 1.02, 1.09; $p < 0.01$) among female recipients. Vitamin D deficiency was associated with a 2.2-fold higher incidence of UTI (aRR = 2.20; 95% CI: 1.57, 3.08; $p < 0.01$) compared to vitamin D sufficiency in male recipients compared to 1.16-fold higher incidence (aRR = 1.16; 95% CI: 0.85, 1.58; $p = 0.35$) in female recipients. The interactions between vitamin D and sex were not significant for other types of infection (Table S3).

TABLE 2 | Association of posttransplant circulating 25(OH)D and late-onset infection.

Incident infection	Baseline serum 25(OH)D[a]			
	≤ 20 ng/mL	21–29 ng/mL	≥ 30 ng/mL	Per 5 ng/mL lower
Overall infection (1448 events)				
No. of events	210	349	889	
IR, per 100 person-years[b]	94 (82, 107)	70 (63, 78)	61 (57, 65)	
Unadjusted RR (95% CI)	**1.53 (1.19, 1.96)**	1.13 (0.92, 1.40)	Reference	**1.08 (1.04, 1.12)**
p value (unadjusted)	**< 0.01**	0.25		**< 0.01**
Adjusted RR (95% CI)	**1.19 (1.00, 1.52)**	0.92 (0.75, 1.14)	Reference	**1.05 (1.01, 1.08)**
p value (adjusted)	**0.05**	0.46		**< 0.01**
Bacterial infection (721 events)				
No. of events	115	167	439	
IR, per 100 person-years[b]	51 (42, 62)	33 (29, 39)	30 (28, 33)	
Unadjusted RR (95% CI)	**1.70 (1.22, 2.35)**	1.10 (0.81, 1.49)	Reference	**1.09 (1.04, 1.15)**
p value (unadjusted)	**< 0.01**	0.53		**< 0.01**
Adjusted RR (95% CI)	1.27 (0.92, 1.76)	0.86 (0.64, 1.17)	Reference	**1.05 (1.00, 1.10)**
p value (adjusted)	0.15	0.35		**0.04**
Urinary tract infection (612 events)				
No. of events	110	141	361	
IR, per 100 person-years[b]	49 (41, 59)	28 (24, 33)	25 (22, 28)	
Unadjusted RR (95% CI)	**1.98 (1.39, 2.82)**	1.13 (0.81, 1.58)	Reference	**1.11 (1.05, 1.18)**
p value (unadjusted)	**< 0.01**	0.46		**< 0.01**
Adjusted RR (95% CI)	**1.59 (1.15, 2.20)**	0.86 (0.62, 1.19)	Reference	**1.07 (1.02, 1.13)**
p value (adjusted)	**< 0.01**	0.36		**< 0.01**
Viral infection (399 events)				
No. of events	53	94	252	
IR, per 100 person-years[b]	24 (18, 31)	19 (15, 23)	17 (15, 20)	
Unadjusted RR (95% CI)	1.37 (0.90, 2.07)	1.07 (0.79, 1.46)	Reference	**1.06 (1.01, 1.12)**
p value (unadjusted)	0.14	0.67		**0.02**
Adjusted RR (95% CI)	1.18 (0.79, 1.77)	0.96 (0.70, 1.30)	Reference	1.05 (0.99, 1.10)
p value (adjusted)	0.42	0.78		0.07
Fungal infection (67 events)				
No. of events	6	18	43	
IR, per 100 person-years[b]	3 (1, 6)	4 (2, 6)	3 (2, 4)	
Unadjusted RR (95% CI)	0.90 (0.37, 2.19)	1.21 (0.61, 2.40)	Reference	1.01 (0.93, 1.11)
p value (unadjusted)	0.83	0.59		0.78
Adjusted RR (95% CI)	0.70 (0.25, 1.91)	1.00 (0.46, 2.19)	Reference	1.00 (0.89, 1.11)
p value (adjusted)	0.48	0.99		0.91

Note: Marginal proportional rates models were fitted to model the incidence rate of infection within 365 days of baseline measurement of serum 25(OH)D. All recurrent infections were taken into account. Models adjusted for recipient demographics (age at transplant, sex, race), recipient body mass index (BMI) at transplant, cause of end-stage kidney disease, donor status, delayed graft function, prior kidney transplant, human leukocyte antigens (HLA)-mismatch, induction and maintenance immunosuppression, history of infection before baseline, transplant year, measurement time of serum 25(OH)D (i.e., months after transplant), season of 25(OH)D measurement, concurrent urine protein–creatinine ratio (UPCR), serum albumin, and estimated glomerular filtration rate (eGFR). Pretransplant CMV serology was further adjusted for viral infection. Statistically significant results were marked in bold.

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; IR, incidence rate; RR, rate ratio.

[a]Baseline serum 25(OH)D measured during 5–13 months after transplantation.

[b]All recurrent infection episodes were taken into account.

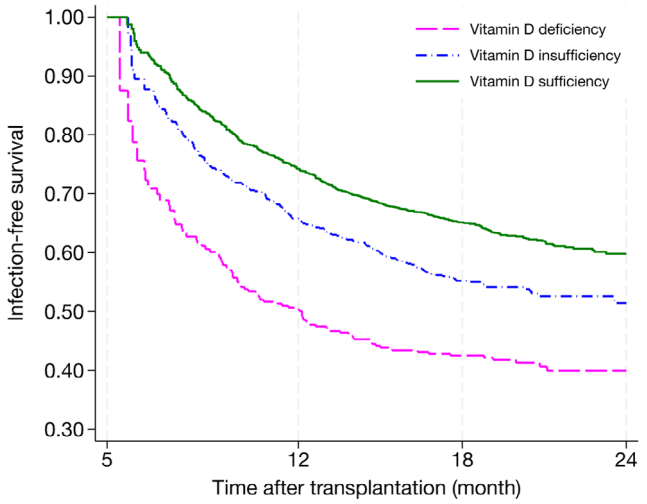

FIGURE 1 | Infection-free survival within one year of baseline window by posttransplant vitamin D status. Vitamin D deficiency: ≤ 20 ng/mL (50 nmol/L); vitamin D insufficiency: 21–29 ng/mL (52.5–72.5 nmol/L); vitamin D sufficiency: ≥ 30 ng/mL (75 nmol/L). Infection episodes within 1 year of baseline window (i.e., 5–13 months posttransplant) were analyzed.

3.6 | Sensitivity Analyses Using Time-Varying 25(OH)D Level

A median of seven posttransplant 25(OH)D measurements (IQR: 4, 10) were included in analyses for each recipient. A total of 2251 infection episodes in 931 recipients were identified in 1 year after each posttransplant 25(OH)D measurement. Bacterial infections accounted for approximately 55%, and UTIs accounted for 45%. Associations between 25(OH)D and infections were generally stronger or similar in these analyses compared to our primary analyses using the first eligible 25(OH)D measurement (Table S4). A stronger association for bacterial infection was detected in analyses using time-varying 25(OH)D levels. Each 5 ng/mL lower 25(OH)D level was associated with a 7% higher incidence of bacterial infection (aRR = 1.07; 95% CI: 1.04, 1.10; $p < 0.01$). The association of 25(OH)D and viral infection remained significant after adjustment in analyses of time-varying 25(OH)D levels, however, with each 5 ng/mL level associated with a 4% higher incidence (aRR = 1.04; 95% CI: 1.00, 1.07; $p = 0.04$). A significant association of 25(OH)D and fungal infection was detected in analyses using time-varying 25(OH)D levels (Table S4). Each 5 ng/mL lower 25(OH)D level was associated with a 7% higher incidence of fungal infection (aRR = 1.07; 95% CI: 1.00, 1.14; $p = 0.05$) after adjustment.

4 | Discussion

Late-onset infection carries an excess burden of hospitalization and threatens long-term survival among KTRs. We found that a lower posttransplant circulating 25(OH)D level is independently associated with a higher incidence of overall late-onset infection, especially UTI. The relationship was shown to be approximately linear without any apparent threshold. Our study sheds light on the potential causal relationship of vitamin D with late-onset infection after kidney transplantation.

Bacterial infections accounted for nearly half of late-onset infections, and UTI was the most common infection in our study, consistent with previous reports [27–32]. A consistently strong association was detected between serum 25(OH)D level and the incidence of bacterial infection, especially UTI, across different analyses. UTI is a common infection among KTRs. An estimated 4%–72% of recipients who experience one UTI episode will

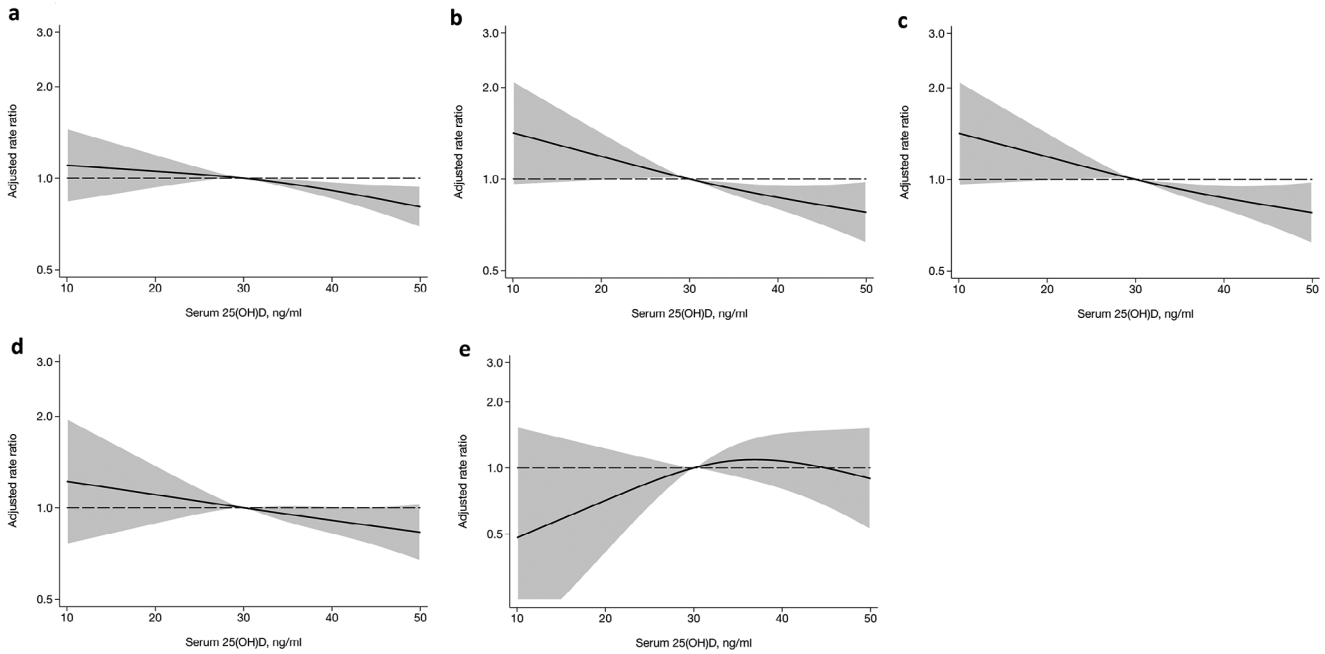

FIGURE 2 | Adjusted rate ratio of incident late-onset infection by posttransplant circulating 25(OH)D level. (a) Overall infection; (b) bacterial infection; (c) urinary tract infection; (d) viral infection; (e) fungal infection. Time-varying circulating 25(OH)D level was fitted using natural cubic spline. Adjusted rate ratio was centered at a 25(OH)D level of 30 ng/mL.

develop recurrent UTI as reported in previous studies [33–36]. It has been recognized that recurrent UTI can undermine allograft function and survival [5–7]. Untreated UTI can lead to allograft pyelonephritis, which requires initial hospitalization and prolonged treatment. Antibiotic prophylaxis (e.g., trimethoprim–sulfamethoxazole) is usually recommended to use for at least 6 months posttransplant, whereas lifelong prophylaxis remains in debate [12]. Our study highlights the need to investigate the use of vitamin D in posttransplant UTI prevention, although we were not able to differentiate specific types of UTI.

Vitamin D plays multiple roles in both innate and adaptive immune processes. Vitamin D receptors (VDR) and 1-α-hydroxylase (which converts inactive 25(OH)D to active $1,25(OH)_2D$) are both found in a variety of immune cells, including activated CD4+ and CD8+ T lymphocytes, dendritic cells, and macrophages [37]. VDR directly regulates the transcription of genes encoding pattern recognition receptors and antimicrobial peptides [17–20]. The balance of T-helper (Th) cells is also influenced by vitamin D, including the differentiation of T-regulatory (Treg) cells and increasing the production of Treg cytokines [38]. By modulating Th cells' balance and enhancing the development of Treg, active vitamin D contributes to protection against pathogens. Fernández-Ruiz et al. observed a positive relationship between levels of serum 25(OH)D at Month 6 posttransplant and CMV-specific mediated immunity (measured by type-II interferon production and CD8+ T cells) in a cohort of KTRs [39]. Moreover, it has been revealed in vitro and in vivo studies that $1,25(OH)_2D$ not only plays a role as a local immunoregulator but also strengthens the bladder epithelial barrier in host resistance against UTI [40–43].

Numerous clinical trials have been performed to evaluate the efficacy of vitamin D supplements in infectious diseases among the general population, with inconsistent results [23, 22]. Previous observational studies observed a significant association between circulating 25(OH)D level and infection in KTRs. Kalluri et al. found that adequate levels of vitamin D in KTRs were associated with lower infection risk in the first year and at any time posttransplantation [28]. Fernández-Ruiz et al. conducted a prospective cohort study where serum 25(OH)D levels were quantified at Months 1, 3, 6, and 12 after transplantation. It was found that vitamin D deficiency (25(OH)D < 12 ng/mL) at Month 1 was an independent risk factor for overall and opportunistic infection but not for bacterial infection [29]. A trial of vitamin D supplementation in renal transplant recipients did not find a significant difference in the incidence of infection between vitamin D supplement low-dose group and the high-dose group [11]. This null finding may be a result of limited statistical power to detect the effect of high dose versus low dose.

The observed associations of lower 25(OH)D and viral and fungal infections were generally less strong than associations with bacterial infections but were statistically significant in some analyses. The risk of opportunistic infections such as CMV and BKPyV infection decreases significantly after 6 months posttransplant. We did not account for the risk stratification based on recipient and donor serology. The analyses for fungal infection were limited by a small sample size, which reduced the statistical power to detect any association.

Our stratified analyses indicate that the association between lower 25(OH)D and UTI is stronger in male recipients as compared to female recipients. We found that the average age of UTI among male recipients was 8 years older than that among females (61 years old in males versus 53 years old in females). The incidence of UTI increases as men age [41]. UTI in older men is underrecognized since women are generally more susceptible due to anatomy. Functional or structural urologic abnormalities (e.g., prostatic hyperplasia) might arise and result in ureteral obstruction. The exposure to urinary catheters post-surgery and immunosuppressants further aggravates the problem. Our study suggests that the potential benefits of vitamin D supplementation for UTI prevention should be explored in older male recipients. Similar results have been reported in other studies. Jorde et al. analyzed data from a randomized clinical trial in patients with prediabetes [45]. A significantly lower incidence of UTI was observed in the group receiving vitamin D supplementation compared to placebo. They found that the association was significant in men, whereas it was not significant in women. An ancillary study of men aged ≥ 55 years in VITAL (VITamin D and OmegA-3 TriaL) found that older men with baseline serum 25(OH)D < 20 ng/mL using vitamin D supplements had lower odds of overactive bladder compared to placebo [46]. These findings of the interaction between vitamin D and sex are consistent with our findings, highlighting the potential use of vitamin D in UTI prevention among male recipients.

Our study has several limitations. The observational study design may allow some residual confounding. We observed that approximately 33% of recipients had vitamin D inadequacy in this cohort despite the vitamin D supplementation of 2000 IU per day prescribed at the time of transplantation. The underlying driver remains unknown with incomplete data of vitamin D supplementation during follow-up. Medication nonadherence might be an explanation. It could also be a result of the resistance to vitamin D repletion. Low 25(OH)D levels may also be a surrogate marker of the degree of immunosuppression. We found that KTRs who received transplants after 2010 were more likely to be included in our study (Table S5). Other baseline characteristics were comparable. We acknowledge the limitation of using a single 25(OH)D level to identify posttransplant vitamin D status in primary analyses given the fact that most recipients have only one valid 25(OH)D level during the baseline window. We took account of the granularity of vitamin D status during follow-up by using time-varying 25(OH)D levels in sensitivity analyses and adjusted for the delayed entry due to nonuniform measurement time. The results of these analyses confirmed the analyses using a single baseline measurement. We acknowledge that there is potential for inflation of type I error given the multiple comparisons. However, the consistency across different analyses provides some confidence in the validity of the results. Results of viral and fungal infections require cautious interpretation due to the limited sample size. We acknowledge the limitation of marginal proportional rates models. The interpretation is only limited to marginal differences in incidence without specification of correlation among recurrent events.

Our study also has noteworthy strengths. Specifically, it benefits from a large sample size and excellent length and completeness of longitudinal follow-up. This is, to our knowledge, the largest number of infections to be analyzed with regards to an

association with 25(OH)D levels in KTRs. The timing of the measurements used is also a notable strength. Fernández-Ruiz et al. showed that levels of serum 25(OH)D increase significantly in the first 6 months after transplantation and then stabilize. Hence, measurement in the baseline window (i.e., 5–13 months posttransplant) should be more representative of posttransplant vitamin D status and indicate the vulnerability to consistent vitamin D inadequacy due to resistance to repletion. In addition, we applied marginal proportional rates models to take account of recurrent infection episodes, whereas previous studies have been limited to the first infection episode as the event of interest. In addition, we performed analyses using both baseline and time-varying circulating 25(OH)D levels to evaluate the consistency across different analyses. We limited the events of interest to those occurring within 1 year after a 25(OH)D measurement to ensure a closer temporal relationship instead of including any infection years afterward.

In conclusion, posttransplant circulating 25(OH)D levels are significantly associated with the incidence of late-onset infection among KTRs. A particularly strong association was observed for the incidence of UTI in male recipients. Our study highlights the need of further studies to determine their causal relationship and underlying mechanisms.

Author Contributions

Zhongyu Yuan: conceptualization, methodology, software, data curation, investigation, validation, formal analysis, visualization, writing – original draft, writing – review and editing. **Michal L. Melamed**: methodology, writing – review and editing, investigation. **Sandesh Parajuli**: methodology, investigation, writing – review and editing. **Didier Mandelbrot**: methodology, investigation, writing – review and editing. **Brad C. Astor**: methodology, conceptualization, investigation, data curation, supervision, funding acquisition, project administration, resources, writing – review and editing.

Ethics Statement

This study was approved by the University of Wisconsin Health Sciences Institutional Review Board and adhered to the Declaration of Helsinki. The clinical and research activities reported were consistent with the Principles of the Declaration of Istanbul on Organ Trafficking and Transplant Tourism. Due to the nature of this study, informed consent specific to this research was not obtained from patients.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The University of Wisconsin Health Sciences Institutional Review Board restricts sharing of clinical data. A request for access to the data used in this study should be sent to the corresponding author, WI.

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Supporting Information

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