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Transferrin Saturation Can Serve as a Novel Biomarker for Predicting the Occurrence and Development of BK Virus-Related Nephropathy After Kidney Transplantation

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ABSTRACT

BK polyomavirus reactivation is a common complication after kidney transplantation, affecting the long-term survival of the transplanted kidney. However, it is unclear whether iron levels affect BKPyV reactivation after kidney transplantation. We first found that preoperative transferrin saturation levels in renal transplant recipients were closely associated with post-transplant BK virus reactivation and progression.

1 | Introduction

BK polyomavirus (BKPyV) reactivation is a common complication after kidney transplantation, affecting the long-term survival of the transplanted kidney. Research indicates that primary BKPyV infection typically presents in childhood, with a median onset age of 4–5 years, and the global seroprevalence among adults stands at approximately 75% (with a range of 46%–94%) [1–4]. Approximately 30%–50% of kidney transplant recipients experience reactivation of latent polyomavirus within the genitourinary tract, leading to subsequent development of BKPyV viremia (BKPyV viremia) in 30%–40% of these individuals, with 20%–40% progressing to histologically confirmed BK virus-associated nephropathy (BKPyVAN) [5–8], ultimately leading to 24%–60% of transplant failures [9, 10]. Despite the known prevalence of BKPyV reactivation after kidney transplantation, reliable biomarkers for predicting its occurrence and progression remain scarce. Identifying such biomarkers is critical for timely intervention and improving long-term graft survival. This study seeks to address this gap by investigating the role of iron metabolism, specifically focusing on transferrin saturation (TSAT), as a potential predictive biomarker for BKPyV infection and nephropathy.

For DNA viruses, the viral genome must be transported into the cell nucleus, and these processes require iron. Efficient viral replication in host cells necessitates an iron-rich environment. The correlation between elevated iron levels and viral activity is commonly observed in various human infectious diseases. It has been confirmed that iron metabolism levels and related derivatives exert varying degrees of influence on viruses such as HIV, HBV, and HPV [11, 12]. However, it remains unclear

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whether iron levels affect BKPyV activation and proliferation in kidney transplant recipients. To our knowledge, this study is the first to evaluate preoperative TSAT as a biomarker for predicting BKPyV infection and its progression to BKPyVAN. By developing and validating a novel nomogram prediction model, we aim to provide clinicians with an accessible tool for individualized risk assessment in kidney transplant recipients, thereby aligning with the current trend toward personalized medicine.

2 | Materials and Methods

2.1 | Patient Populations and Data Collection

This retrospective analysis was conducted utilizing clinical data from recipients who underwent allogeneic kidney transplantation at the First Affiliated Hospital of Zhengzhou University, China, spanning from September 15, 2019, to September 15, 2023. Inclusion criteria for the cohort were as follows: (1) Detection of serum iron-related markers within 1 week before transplantation; (2) Regular follow-up of recipients post-transplantation with urine and/or blood BK viral load detected by fluorescence quantitative PCR. Exclusion criteria comprised: (1) second transplantation, double kidney transplantation, or transplanted kidney failure; (2) Absence of relevant indicator detection or missing follow-up data. The study's endpoints were defined as the diagnosis of BKPyV viremia or BKPyVAN during the follow-up period. The study protocol was approved by the ethics review committee of our center (batch number: 2021-KY-0781-001). Given the retrospective nature of this study, additional informed consent from patients was deemed unnecessary.

2.2 | Baseline Data Collection

(1) Recipient baseline data encompass gender, age, body mass index (BMI), dialysis history, cold ischemia time, blood type matching, human leukocyte antigen (HLA) matching, and other relevant factors. (2) Immunosuppressive treatment regimen: Patients receive immunosuppression induction with either anti-thymocyte globulin (ATG) or basiliximab in combination with methylprednisolone. Post-surgery, the fundamental immunosuppressive treatment regimen comprises tacrolimus and mycophenolate mofetil (MMF), considering glucocorticoid usage based on the patient's condition. (3) Recipient's preoperative markers of iron metabolism, including serum iron, ferritin, unsaturated iron binding capacity (UIBC), total iron binding capacity (TIBC), and TSAT, among others. (4) Adverse event documentation: Record occurrences of adverse events such as delayed graft function (DGF) and acute rejection (AR) during the perioperative period.

2.3 | Diagnostic Criteria for BKPyV Reactivation

Urine screening for BKPyV is conducted biweekly during the initial 3 months post-transplantation, transitioning to monthly screenings until the 6th month, followed by screenings every 3 months until 2 years post-transplantation. In the event of a positive urine screen, blood screening for BKPyV is initiated. Plasma testing is performed symptomatically or monthly during the first 6 months, with subsequent screenings every 3 months until 2 years post-transplantation. BKPyVemia is defined as plasma BKPyV-DNA exceeding >1000 copies/mL for more than 3 weeks or any blood BKPyV-DNA load surpassing >104 copies/mL[6]. Recipients meeting these criteria undergo pathological biopsy, with BK nephropathy diagnosed based on the Banff classification [13].

2.4 | DGF Diagnostic Criteria

Diagnosis of DGF can be established if any of the following criteria are met: (1) Requirement for hemodialysis at least once within the week post-surgery. (2) Serum creatinine level exceeding 400 μmol/L on the 10th day post-surgery. (3) Serum creatinine level surpassing 300 μmol/L on the 14th day post-surgery [14, 15], with exceptions made for elevated serum creatinine levels attributable to early AR or nephrotoxicity induced by immunosuppressants.

2.5 | AR Diagnostic Criteria

Upon exclusion of vascular or ureteral complications, which typically manifest as postoperative decreased urine output, elevated serum creatinine, and graft swelling or tenderness, additional investigations were conducted. Color Doppler ultrasonography of the graft revealed an increase in arterial vascular resistance index >0.75 at any level, indicative of a favorable response to pulse therapy. The pathological diagnosis adhered to the Banff guidelines [16], with biopsy results aligning with the histological criteria for diagnosing AR.

2.6 | Statistical Analysis

Categorical variables are reported as percentages, while continuous variables are presented as $\bar{x} \pm S$ or quartiles, depending on their distribution. Predictors were identified based on clinical importance, scientific knowledge, and predictors identified in previously published articles. The COX proportional hazards regression model was used to assess the relationship between relevant variables and either BKPyV viremia or BKPyVAN. Variables with a univariate p-value <0.1 were included in the multivariate COX regression analysis, and a stepwise selection method was employed to screen variables in the multivariate analysis [17]. Selected variables were incorporated in the nomograms to predict the probability of 6-month and 1-year BKPyV reactivation rates after kidney transplantation using statistical software (rms in R, version 4.2.0; http://www.r-project.org) [18]. The nomogram is based on proportionally converting each regression coefficient in multivariate logistic regression to a 0- to 180-point scale. The effect of the

variable with the highest β coefficient (absolute value) is assigned 180 points. The points are added across independent variables to derive total points, which are converted to predicted probabilities. Evaluate model performance through discrimination, model fitting effect, and clinical application efficacy. The performance of the nomograms was evaluated using the C statistics. The C statistic estimates the probability of concordance between predicted and observed outcomes in rank order and is equivalent to the area under the receiver operating characteristic curve. Calibration was evaluated using a calibration plot, a graphic representation of the relationship between the observed outcome frequencies and the predicted probabilities. In a well-calibrated model, the predictions should fall on a 45° diagonal line. The clinical application efficacy of the model was evaluated through decision curve analysis (DCA) and tested in the validation cohort. Two-sided test, $p < 0.05$ was considered to be statistically significant.

3 | Results

3.1 | Demographic and Epidemiological Characteristics

A total of 1546 cases of kidney transplant surgeries were collected from September 15, 2019, to September 15, 2023. As depicted in Figure 1, among these cases, 821 patients had missing serum iron-related markers testing or incomplete testing items before transplantation, while 36 cases were lacking post-transplantation urine/blood BK virus load testing. Additionally, 12 cases involved secondary transplantation, bilateral kidney transplantation, or transplant kidney failure. Furthermore, six cases had missing relevant predictive factors or follow-up data. After excluding these cases, a total of 626 recipients meeting the criteria were included for analysis. The baseline characteristics of these 626 recipients are detailed in Table 1. Among them, 38 cases (6.1%) exhibited confirmed BK viremia and 18 cases (2.9%) were diagnosed with biopsy-proven BK nephropathy. The median follow-up time was 9.3 months (range 0.1–64.2 months). The incidence rates of BKPyV viremia at 6 months and 1 year post-renal transplantation were 4.2% and 5.3%, respectively. Similarly, the incidence rates of BKPyVAN at 6 months and 1 year post-transplantation were 1.6% and 2.4%, respectively.

3.2 | Analysis of the Correlation Between Iron Levels and BK Virus Reactivation After Renal Transplantation

Univariate COX regression was used to analyze the association of kidney transplant recipient-related markers with BK

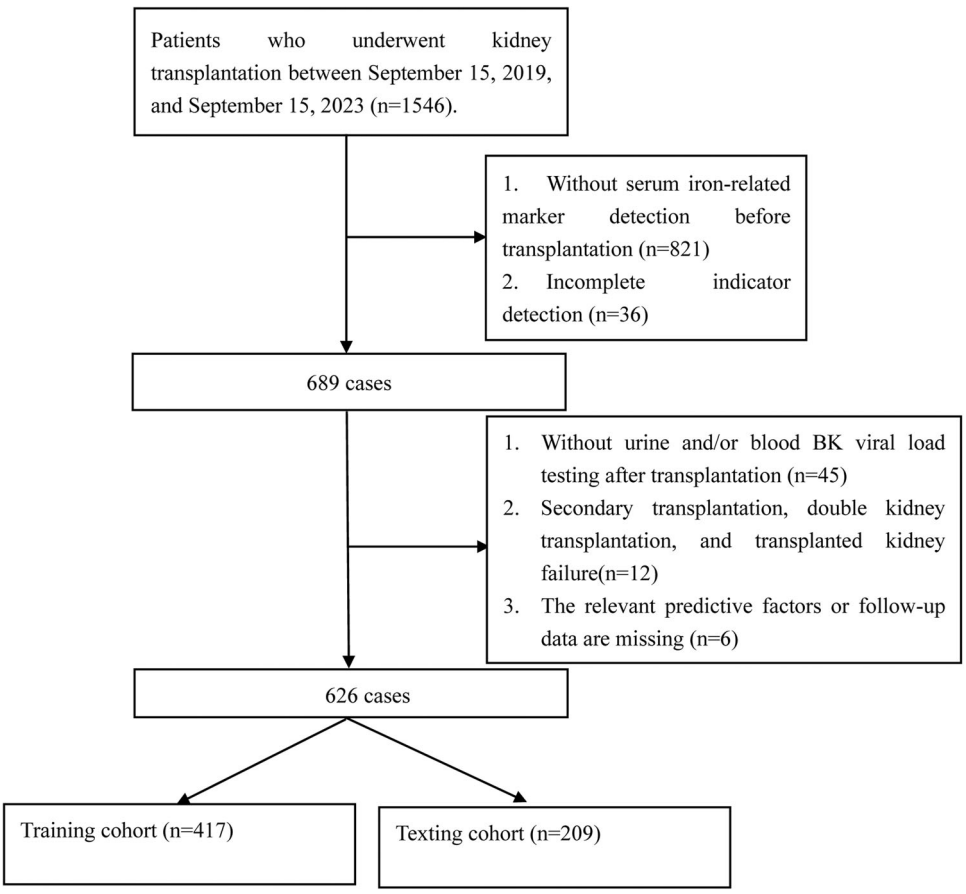

FIGURE 1 | Case screening flow chart.

TABLE 1 | Baseline data of 626 recipients.

Characteristic	Total ($n = 626$)
Age, y, mean (SD)	32.2 (11.9)
Sex	
Female	201 (32.1)
Male	425 (67.9)
BMI, kg/m^2, mean (SD)	20.7 (2.9)
Iron, μmol/L, median (IQR)	13.9 (10.5–18.3)
Ferritin, μg/L, median(IQR)	190 (76.4–406.5)
UIBC, μmol/L, median(IQR)	28.5 (21.9–36.0)
TIBC, μmol/L, median(IQR)	43.6 (37.9–50.1)
TSAT, %	32.4 (23.1, 43.2)
HLA mismatch, *n*	
≥ 3	341 (54.5)
< 3	285 (45.5)
Preemptive transplantation	
Yes	95 (15.2)
No	531 (84.8)
Coldtime, h, median(IQR)	10.0 (5.8–13.5)
Transplant type	
LKT	155 (24.8)
DCD	471 (75.2)
Treatment plan	
FK + MMF + Pred	508 (81.2)
FK + MMF	118 (18.8)
Bloodmatch	
Yes	610 (97.4)
No	16 (2.6)
Induction	
Basiliximab	70 (11.2)
ATG	556 (88.8)
AR	
Yes	66 (10.5)
No	560 (89.5)
DGF	
Yes	64 (10.2)
No	562 (89.8)

Abbreviations: AR, acute rejection; ATG, anti-thymocyte globulin; BMI, body mass index; DCD, donation after circulatory death; DGF, delayed graft function; FK, FK506; HLA, human leukocyte antigen; LKT, living kidney transplantation; MMF, mycophenolate mofetil; Pred, prednisone; TIBC, total iron binding capacity; TSAT, transferrin saturation; UIBC, unsaturated iron-binding capacity.

viremia and BK virus nephropathy in 626 recipients. The results of single factor analysis are shown in Table 2, UIBC (0.96 [0.94–0.99]), TIBC (0.94 [0.91–0.98]), TSAT(1.02 [1.01–1.04]), BMI (1.20 [1.07–1.35]), cold shortage Blood time (1.08 [1.02–1.13]), transplant type (0.36 [0.13–1.00]), postoperative AR (3.87 [1.94–7.70]) are related to the occurrence of BKV viremia. Preoperative higher levels of TSAT and TIBC in recipients serve as inhibitory factors for the occurrence of post-renal transplant BK viremia. Conversely, preoperative higher levels of TSAT, higher BMI values, longer cold ischemia time of the donor's kidney, deceased donor transplantation compared to living donor transplantation, and postoperative occurrence of AR promote factors for post-renal transplant BK viremia. The analysis results of the correlation between relevant markers and BK virus nephropathy indicate that UIBC (0.95 [0.91–0.99]), TIBC (0.93 [0.88–0.98]), TSAT(1.03 [1.01–1.05]), BMI (1.26 [1.03–1.56]), gender (9.04 [1.20–68.12]), cold ischemia time (1.08 [1.02–1.16]), blood type matching transplantation (0.16 [0.04–0.70]), and postoperative occurrence of AR (3.86 [1.42–10.47]) are associated with the occurrence of BKPyVAN. Preoperative higher levels of UIBC, TIBC, and same blood type kidney transplantation serve as inhibitory factors for the occurrence of post-renal transplant BK virus nephropathy. Conversely, preoperative higher levels of TSAT, higher BMI values, longer cold ischemia time of the donor kidney, male recipients compared to female recipients, and postoperative occurrence of AR serve as promoting factors for post-renal transplant BKPyVAN. These results suggest a correlation between preoperative iron levels and the risk of BK virus reactivation post-renal transplantation. Specifically, higher preoperative iron levels in recipients are associated with an increased risk of BK virus reactivation after renal transplantation.

3.3 | Variable Screening and Risk Ratios

The 626 recipients were randomly allocated into training and validation cohorts at a ratio of 2:1. As shown in Table 3, there were no statistically significant differences in baseline characteristics between the training and validation cohorts. The variables listed in Table 2 were used for univariate COX regression analysis. The results of single factor analysis are shown in Table 4, BMI (1.18 [1.02–1.37]), AR (3.56 [1.53–8.24]), UIBC (0.95 [0.91–0.99]), TIBC (0.94 [0.90–0.97]), TSAT (1.02 [1.00–1.05]), and so on, are related to the occurrence of BKV viremia, BMI (1.26 [1.03–1.56]), AR (5.19 [1.60–16.77]), UIBC (0.94 [0.89–0.99]), and so on. are related to BKPyVAN occurrence related. As shown in Table 5, stepwise screening in the multifactor COX proportional hazards regression model identified the following four variables that were independently associated with the occurrence of BKPyV viremia, BMI (1.27 [1.08–1.50]), AR (3.68 [1.57–8.66]), cold ischemia time (1.09 [1.02–1.17]), TSAT (1.03 [1.01–1.05]). Similarly, the following five variables were identified independently associated with the occurrence of BKPyVAN, BMI (1.49 [1.14–1.94]), AR (9.84 [2.80–34.62]), cold ischemia time (1.15 [1.05–1.27]), TSAT (1.05 [1.01–1.09]) and male recipients (11.07 [1.11–110.78]).

3.4 | Nomogram and Model Performance

A nomogram chart (Figure 2) was developed to predict BKPyV viremia and BKPyVAN by incorporating the independent risk

TABLE 2 | Univariate COX regression analysis of BKV viremia and proven-BKPyVAN based on perioperative data from a cohort of 626 recipients.

	BK-Viremia		Proven-BKPyVAN	
Variable	**HR (95%CI)**	***p*-value**	**HR (95%CI)**	***p*-value**
Factors selected				
Age, *y*	1.01 (0.99–1.04)	0.406	1.03 (0.99–1.07)	0.223
BMI, kg/m^2	1.20 (1.07–1.35)	0.003	1.29 (1.08–1.53)	0.004
Iron, μmol/L	1.03 (0.99–1.07)	0.115	1.03 (0.97–1.09)	0.308
Ferritin, μg/L	1.00 (1.00-1.01)	0.173	1.00 (0.99–1.01)	0.783
UIBC, μmol/L	0.96 (0.94–0.99)	0.012	0.95 (0.91–0.99)	0.014
TIBC, μmol/L	0.94 (0.91–0.98)	0.003	0.93 (0.88–0.98)	0.005
TSAT, %	10.15 (2.13–48.29)	0.004	15.61 (1.71–142.16)	0.015
Sex				
Male	2.26 (0.99–5.15)	0.053	9.04 (1.20–68.12)	0.033
Female	1 [Reference]	NA	1 [Reference]	NA
HLA mismatch, *n*				
≥ 3	0.61 (0.32–1.16)	0.131	0.81 (0.32–2.05)	0.658
< 3	1 [Reference]	NA	1 [Reference]	NA
Preemptive transplantation				
Yes	0.62 (0.29–1.32)	0.213	0.96 (0.28–3.32)	0.947
No	1 [Reference]	NA	1 [Reference]	NA
Coldtime, *h*	1.08 (1.02–1.13)	0.004	1.08 (1.02–1.16)	0.017
Transplant type				
LKT	0.36 (0.13–1.00)	0.050	0.62 (0.18–2.15)	0.452
DCD	1 [Reference]	NA	1 [Reference]	NA
Treatment plan				
FK + MMF + Pred	0.82 (0.25–2.67)	0.741	1.21 (0.28–5.30)	0.800
FK + MMF	1 [Reference]	NA	1 [Reference]	NA
Bloodmatch				
Yes	0.35 (0.08–1.45)	0.147	0.16 (0.04–0.70)	0.015
No	1 [Reference]	NA	1 [Reference]	NA
Induction				
Basiliximab	0.66 (0.26–1.70)	0.393	0.91 (0.26–3.15)	0.882
ATG	1 [Reference]	NA	1 [Reference]	NA
AR				
Yes	3.87 [1.94–7.70]	0.000	3.86 (1.42–10.47)	0.008
No	1 [Reference]	NA	1 [Reference]	NA
DGF				
Yes	2.00 [0.88–4.55]	0.099	1.84 (0.53–6.36)	0.335
No	1 [Reference]	NA	1 [Reference]	NA

Abbreviations: AR, acute rejection; ATG, anti-thymocyte globulin; BMI, body mass index; DCD, donation after circulatory death; DGF, delayed graft function; FK, FK506; HLA, human leukocyte antigen; LKT, living kidney transplantation; MMF, mycophenolate mofetil; Pred, prednisone; TIBC, total iron binding capacity; TSAT, transferrin saturation; UIBC, unsaturated iron-binding capacity.

factors outlined in Table 5. Each variable in the Nomogram corresponds to a specific point value. By summing up the points corresponding to all variables, the risk of developing BKPyV viremia or BKPyVAN at postoperative 6 months, 1 year, and 2 years can be directly calculated.

For instance, consider a recipient with a BMI of 24 kg/m^2, preoperative TSAT of 0.4, kidney transplant cold ischemia time of 10 h, and postoperative occurrence of AR, resulting in matching points of 72, 20, 16, and 25 for each respective variable. The total sum of points would be 133. Thus, the

TABLE 3 | Baseline characteristics of training cohort and validation cohort.

Variable	Cohort		*p*-value
	Training ($n = 417$)	Validation ($n = 209$)	
Sex			
Male	281 (67.7)	144 (32.3)	0.702
Female	136 (66.1)	65 (33.9)	
HLA match			
≥ 3	230 (65.6)	111 (34.4)	0.628
< 3	187 (67.4)	98 (32.6)	
Preemptive transplantation			
Yes	71 (74.7)	24 (25.3)	0.068
No	346 (65.2)	185 (34.8)	
Blood type matching			
Yes	408 (66.9)	202 (33.1)	0.373
No	9 (56.3)	7 (43.7)	
transplant type			
DCD	315 (66.9)	156 (33.1)	0.806
LRRT	102 (65.8)	53 (34.2)	
Induction			
ATG	342 (67.3)	166 (32.7)	0.435
Basiliximab	75 (63.6)	43 (36.4)	
Treatment plan			
ATG + MMF + Pred	374 (67.3)	182 (32.7)	0.329
ATG + MMF	43 (61.4)	27 (38.6)	
AR			
Yes	48 (72.7)	18 (27.3)	0.265
No	369 (65.9)	191 (34.1)	
DGF			
Yes	45 (70.3)	19 (29.7)	0.508
No	372 (66.2)	190 (33.8)	
Age, mean (SD), *y*	32.5 (11.0)	31.5 (13.6)	0.324
BMI, mean (SD), kg/m^2	20.9 (2.7)	20.4 (3.1)	0.063
Coldtime, mean (SD), *h*	9.1 (5.6)	9.9 (5.4)	0.094
Iron, mean (SD), μmol/L	15.1 (6.4)	15.4 (8.9)	0.608
Serum ferritin, *M*(*Q*1, *Q*3), μg/L	213.9 (80.8, 424.8)	159.9 (71.4, 384.4)	0.066
UIBC, mean (SD), μmol/L	29.3 (13.0)	31.1 (14.8)	0.113
TIBC, mean (SD), μmol/L	44.4 (11.3)	46.3 (11.5)	0.052
TSAT, mean (SD), %	35.5 (16.1)	35.3 (21.1)	0.934

Abbreviations: AR, acute rejection; ATG, anti-thymocyte globulin; BMI, body mass index; DCD, donation after circulatory death; DGF, delayed graft function; FK, FK506; HLA, human leukocyte antigen; LKT, living kidney transplantation; MMF, mycophenolate mofetil; Pred, prednisone; TIBC, total iron binding capacity; TSAT, transferrin saturation; UIBC, unsaturated iron-binding capacity.

probability of developing BKPyV viremia at 6 months, 1 year, and 2 years post-transplantation would be approximately 0.78, 0.70, and 0.65, respectively, as illustrated in Supplementary Material S1. This calculation method is equally applicable to the BKPyVAN Nomogram model.

3.5 | Model Performance Evaluation

A time-dependent ROC curve (Figure 3) was generated to assess the predictive accuracy of the Nomogram model at various time points following surgery. The Nomogram exhibited strong

TABLE 4 | Univariate COX regression analysis of BKV viremia and Proven-BKPyVAN based on perioperative period data in the training cohort.

	BK-Viremia		Proven-BKPyVAN	
Variable	**HR (95%CI)**	***p*-value**	**HR (95%CI)**	***p*-value**
Factors selected				
Age, *y*	1.01 (0.98–1.05)	0.565	1.02 (0.97–1.07)	0.519
BMI, kg/m^2	1.18 (1.02–1.37)	0.023	1.26 (1.03–1.56)	0.028
Iron, μmol/L	1.03 (0.97–1.09)	0.380	1.04 (0.96–1.12)	0.405
Ferritin, μg/L	1.00 (1.00–1.01)	0.145	1.00 (0.99–1.01)	0.957
UIBC, μmol/L	0.95 (0.91–0.99)	0.009	0.94 (0.89–0.99)	0.044
TIBC, μmol/L	0.94 (0.90–0.97)	0.011	0.94 (0.88–1.00)	0.065
TSAT, %	11.27 (1.38–92.04)	0.024	18.72 (0.93–376.89)	0.056
Sex				
Male	2.20 (0.82, 5.86)	0.116	6.05 (0.78–47.07)	0.086
Female	1 [Reference]	NA	1 [Reference]	NA
HLA match, *n*				
≥ 3	0.58 (0.27–1.27)	0.172	0.54 (0.17–1.70)	0.289
< 3	1 [Reference]	NA	1 [Reference]	NA
Preemptive transplantation				
Yes	0.49 (0.21–1.13)	0.095	0.64 (0.17–2.36)	0.497
No	1 [Reference]	NA	1 [Reference]	NA
Coldtime, *h*	1.06 (1.00–1.12)	0.058	1.08 (1.00–1.16)	0.072
Transplant type				
LKT	0.41 (0.12–1.38)	0.152	0.67 (0.15–3.06)	0.601
DCD	1 [Reference]	NA	1 [Reference]	NA
Treatment plan				
ATG + MMF + Pred	0.88 (0.21–3.74)	0.864	0.98 (0.13–7.70)	0.988
ATG+MMF	1 [Reference]	NA	1 [Reference]	NA
Bloodmatch				
Yes	0.41 (0.06–3.05)	0.383	0.18 (0.02–1.42)	0.104
No	1 [Reference]	NA	1 [Reference]	NA
Induction				
Basiliximab	0.67 (0.16–2.83)	0.584	1.75 (0.38–8.00)	0.470
ATG	1 [Reference]	NA	1 [Reference]	NA
AR				
Yes	3.56 [1.53–8.24]	0.003	5.19 (1.60–16.77)	0.006
No	1 [Reference]	NA	1 [Reference]	NA
DGF				
Yes	2.01 [0.76–5.35]	0.161	3.00 (0.81–11.08)	1.000
No	1 [Reference]	NA	1 [Reference]	NA

Abbreviations: AR, acute rejection; ATG, anti-thymocyte globulin; BMI, body mass index; DCD, donation after circulatory death; DGF, delayed graft function; FK, FK506; HLA, human leukocyte antigen; LKT, living kidney transplantation; MMF, mycophenolate mofetil; Pred, prednisone; TIBC, total iron binding capacity; TSAT, transferrin saturation; UIBC, unsaturated iron-binding capacity.

discrimination in predicting the risk of BKPyV viremia and BKPyVAN. The C-statistics for predicting the occurrence of BKPyV viremia at 6 months and 1 year postoperatively were 0.77 (95% CI, 0.68–0.86) and 0.80 (95% CI, 0.72–0.88), respectively. Similarly, in the validation cohort, the Nomogram models displayed excellent discriminative performance, with C-statistics of 0.83 (95% CI, 0.72–0.95) at 6 months and 0.86 (95% CI, 0.75–0.97) at 1 year postoperatively.

TABLE 5 | Multivariate COX regression analysis of BKV viremia and proven-BKPyVAN based on perioperative period data in the training cohort.

	BK viremia		Proven-BKPyVAN	
Variable	**HR (95%CI)**	***p*-value**	**HR (95%CI)**	***p*-value**
BMI, kg/m^2	1.27 (1.08–1.50)	0.004	1.49 (1.14–1.94)	0.003
coldtime	1.09 (1.02–1.17)	0.014	1.15 (1.05–1.27)	0.004
TSAT, %	14.84 (1.97–111.61)	0.009	101.35 (2.92–3512.12)	0.011
Gender				
Male	NA	NA	11.07 (1.11–110.78)	0.041
Female	NA	NA	1 [Reference]	NA
AR, yes versus no				
Yes	3.68 (1.57–8.66)	0.003	9.84 (2.80–34.62)	0.000
No	1 [Reference]	NA	1 [Reference]	NA

Abbreviations: AR, acute rejection; BMI, body mass index; NA, not applicable; TSAT, transferrin saturation.

The C-statistics for assessing the occurrence of BKPyVAN at 6 months and 1 year postoperatively were 0.85 (95% CI, 0.75–0.95) and 0.89 (95% CI, 0.82–0.97), respectively. In the validation cohort, the C-statistics for assessing the occurrence of BKPyVAN at 6 months and 1 year postoperatively were 0.91 (95% CI, 0.78–1.05) and 0.93 (95% CI, 0.84–1.03), respectively. Through 1000 iterations of bootstrap validation, the accuracy and potential overfitting of the training model were assessed. The line charts demonstrated good accuracy in estimating the risk of BKPyV viremia and BKPyVAN, with bootstrapped-corrected C-indexes of 0.77 (95% CI, 0.70–0.85) and 0.88 (95% CI, 0.82–0.95), respectively. The uncorrected C-indexes were 0.75 and 0.86.

Additionally, calibration curves were graphically presented in Figure 4, illustrating the 100-sample and 50-sample bootstrap calibration plots for predicting BKPyV viremia and BKPyVAN reactivation at 6 months and 1-year post-transplantation in both the training and validation cohorts. The risk assessment conducted through line charts showed good consistency with clinical pathology and laboratory results.

3.6 | Clinical Use

The decision curve analysis, both without integrating TSAT and with integrated TSAT, was conducted in the training cohort and validation cohort, as illustrated in Figure 5. The decision curve indicated that if the threshold probability of a patient or doctor exceeds 4%, utilizing the radionic Nomogram to predict BKPyV viremia and BKPyVAN offers more benefit than either the treat-all-patients scheme or the treat-none scheme [19]. Within this range, models integrating recipient preoperative TSAT demonstrated a higher net benefit compared to models that did not integrate TSAT.

4 | Discussion

In this study, we identified preoperative TSAT in kidney transplant recipients as an independent risk factor for postoperative BK virus infection. We developed and validated a nomogram prediction model to help assess the risk of BKPyV viremia and BKPyVAN infection in individual recipients. Our results indicate that the model can accurately predict the occurrence and progression of BKPyV viremia and BKPyVAN after kidney transplantation. The calibration curve showed a good fit for the model, and the DCA curve demonstrated that incorporating preoperative TSAT into the nomogram model provides better clinical net benefit. This retrospective study suggests that iron metabolism levels may influence BKPyV replication in recipients.

In this study, we first analyzed the correlation between preoperative extracellular iron levels and postoperative BK virus infection in kidney transplant recipients. The results showed that iron-related indicators, such as UIBC, TIBC, and TSAT, were associated with the occurrence of post-transplant BK viremia and BKPyVAN. After adjusting for confounding biases by incorporating previously confirmed risk factors based on clinical relevance, TSAT remained an independent risk factor for both BK viremia and BK virus nephropathy after kidney transplantation. TSAT and serum ferritin serve as the cornerstone for evaluating iron status [19, 20]. Under normal conditions, only one-third of transferrin in the serum is bound to iron. During iron deficiency, the UIBC increases due to a decrease in iron concentration, while TSAT reflects the amount of iron required to reach the saturated iron-binding capacity of transferrin. The relevant indicators suggest that higher preoperative iron metabolism levels in recipients are associated with BKPyV infection.

Studies have observed an increase in TSAT, serum ferritin, and liver iron concentration in patients infected with HCV [21]. Elevated iron status is also associated with increased mortality in HIV-1 reactivation, as iron accumulation favors HIV-1 replication, leading to a decline in immune function in patients [22, 23]. Additionally, excessive iron storage may decrease HPV clearance rates, potentially stimulating virus activity and

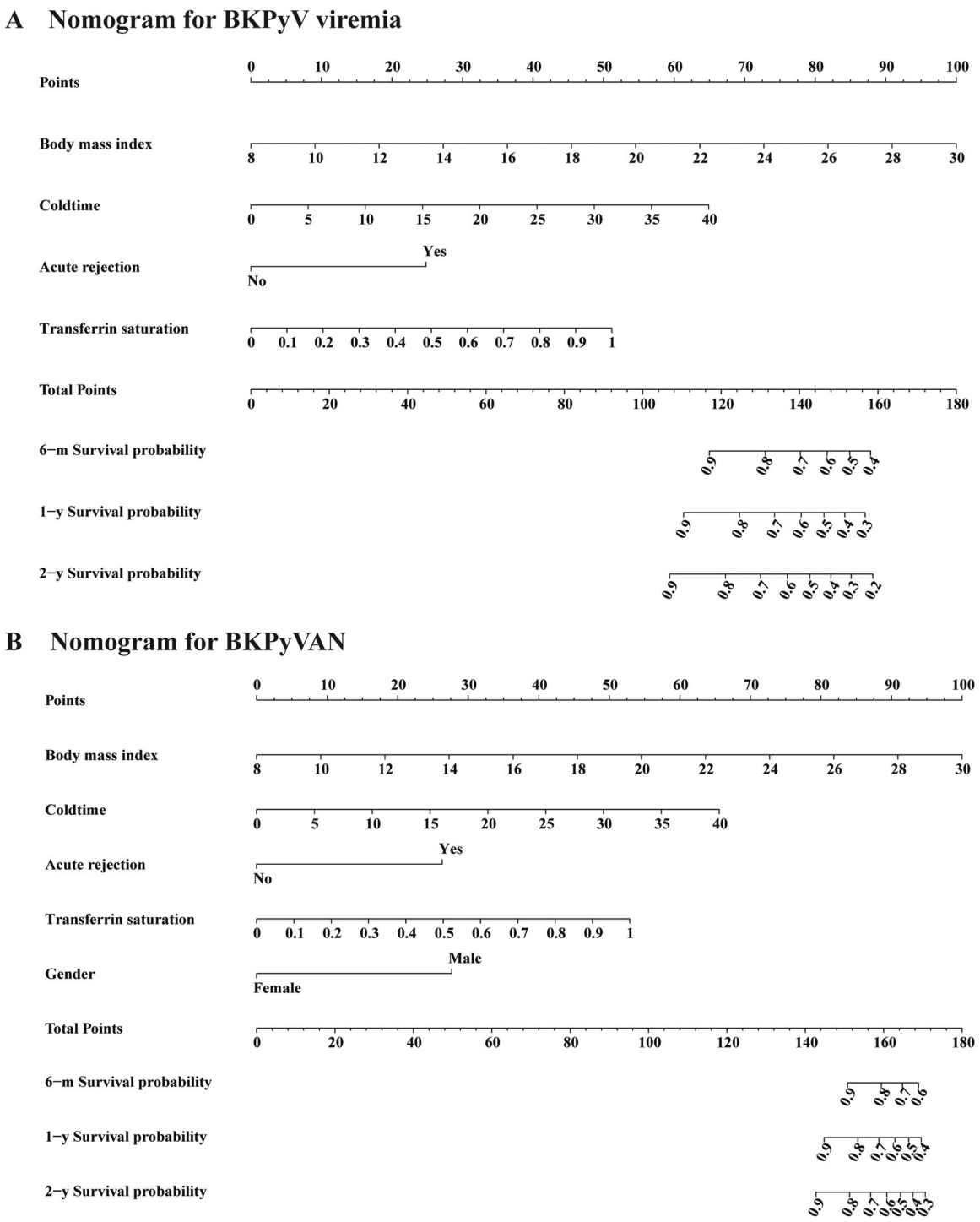

FIGURE 2 | The nomogram to predict BKPyV viremia and BKPyVAN survival was created based on independent prognostic factors in the training cohort. (A) Nomograms for predicting proportion of patients with BKV viremia survival after surgery. (B) Nomograms for predicting proportion of patients with BKPyVAN survival after surgery.

promoting oxidative DNA damage [2]. Elevated iron storage can also lead to the production of reactive oxygen specie, which can promote the transcription of HPV viral proteins E6 and E7, HPV replication, and host cell proliferation [24]. These findings are in line with our discoveries. Consequently, we hypothesize that higher iron metabolism levels may either impede the clearance of BK virus or promote viral replication and transcription of related genes, thereby facilitating virus proliferation within host cells. Specifically, further research is warranted to explore the mechanisms by which extracellular iron influences virus replication and transcription within host cells and whether there exists a synergistic effect between intracellular and extracellular iron.

In our study, no correlation was found between HLA mismatch and the occurrence of BKV viremia and BKPyVAN,

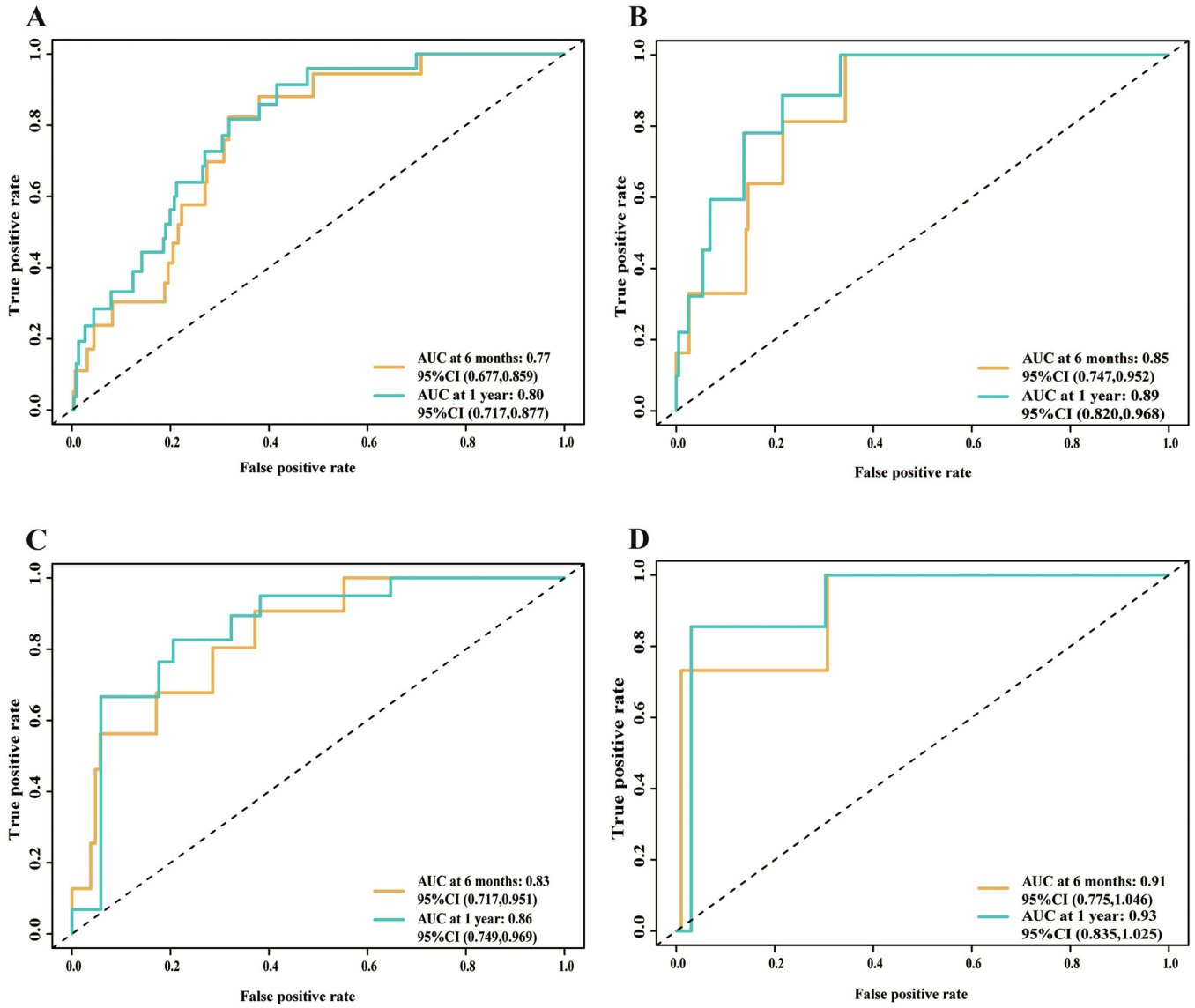

FIGURE 3 | Time-dependent ROC curves compare the prognostic accuracy of the risk factors in training and texting cohort. (A, C) Time-dependent ROC curves by BKPyV viremia nomograms for reactivation probability. (B, D) Time-dependent ROC curves by BKPyVAN nomograms for reactivation probability. (A, B) Comparisons of the prognostic accuracy by the four-based risk factors in training cohort. (C, D) Comparisons of the prognostic accuracy by the five-based risk factors in texting cohort.

which may be related to the type and degree of HLA mismatch. Masutani et al. found that HLA-A2, HLA-B44 and HLA-DR15 mismatch were associated with the presence of viremia, but other types of HLA mismatch did not show statistical differences [25]. In contrast, many studies have not demonstrated any association between overall HLA mismatching and the development of viremia or BKV nephropathy [26, 27]. In fact, in one study, HLA mismatching was associated with a lower incidence of graft loss in patients with BKPyVAN [28]. In addition, we did not find that ATG as an induction regimen increased the incidence of BKV viremia and BKPyVAN compared with basiliximab. This may be related to the dosage we used and the duration of BK virus infection in the recipient. The study by Darshana et al. showed that full-dose ATG induction (five doses of 1.5 mg/kg/day) treatment increased the risk of BK virus infection in renal transplant recipients 6 months after surgery, but single-dose ATG treatment did not find a significant difference [29]. The study by Prince et al. found that ATGAM as an induction therapy rather than ATG was an important risk factor for BKPyVAN [30]. Interestingly, we found that BMI increases the risk of BKPyV infection in recipients after surgery. This may be related to the individualized medication guidance program of our center. Considering the differences in immunity among different patient populations, patients with a relatively large base weight will adopt a relatively aggressive immunosuppression induction and maintenance program, which may increase the risk of BKPyV infection in recipients.

Furthermore, this study represents the pioneering application of the Nomogram model to predict the occurrence and

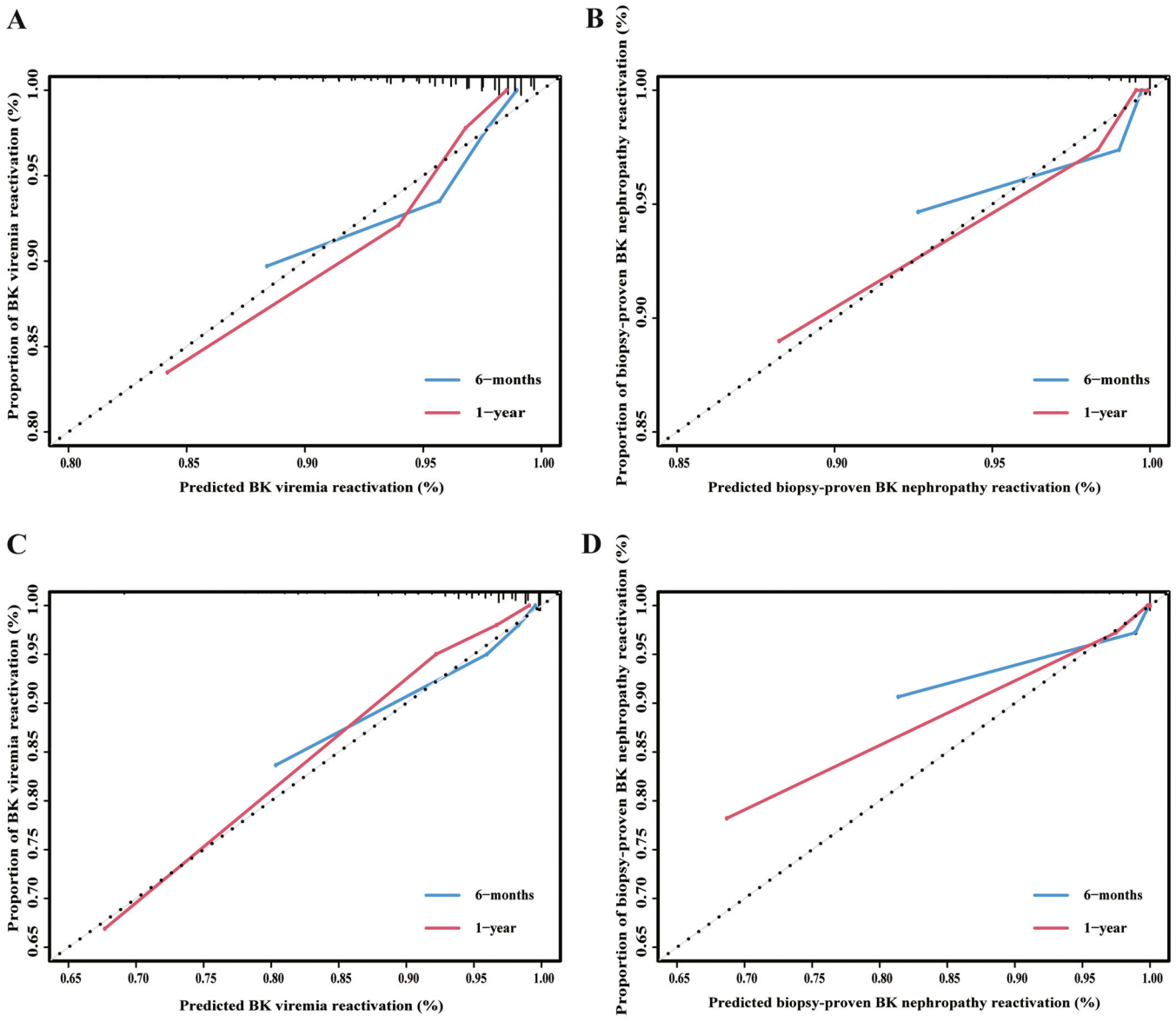

FIGURE 4 | (A–D) Plots depict the calibration of each model in terms of agreement between predicted and observed 6-month, 1-year outcomes. Model performance is shown by the plot, relative to the 45° line, which represents perfect prediction. (A, B) Validity of the predictive performance of the nomogram in estimating the risk of BKPyV viremia and BKPyVAN reactivation in the training cohort ($n = 417$) respectively. (C, D) Validity of the predictive performance of the nomogram in estimating the risk of BKPyV viremia and BKPyVAN reactivation in the validation cohort ($n = 209$) respectively.

progression of BKPyV viremia and BKPyVAN at various intervals following surgery. The postoperative periods of 6 months and 1 year are recognized as high-risk periods for BK virus reactivation [16]. Predicting at different time points offers clinicians more detailed and precise information, facilitating disease monitoring and preventive management. The nomograms constructed in this study for predicting BKPyV viremia and BKPyVAN include four and five comprehensive, easily accessible variables, respectively. These models facilitate individualized prediction of postoperative BKPyV infection, offering economic benefits and aligning with the current trend toward personalized medicine.

In the context of chronic kidney disease (CKD) and hemodialysis, factors such as increased iron loss, reduced iron absorption, and limited iron availability contribute to iron deficiency [31]. Iron therapy plays a crucial role in managing anemia in CKD patients, especially those undergoing hemodialysis. Clinically, various iron preparations are available for oral or intravenous administration. However, uncertainties persist regarding the initiation, optimal dosage, and monitoring of iron therapy, raising concerns about the long-term safety of iron supplementation. Iron levels hold significant implications at different stages of kidney disease progression. Therefore, it is essential to thoroughly investigate the impact of both intracellular and extracellular iron levels on patients with CKD, those undergoing hemodialysis, and kidney transplant recipients.

The limitation of this study is that it is based on a single-center cohort. Further research is needed to establish multicenter, large-sample clinical cohorts for prospective validation.

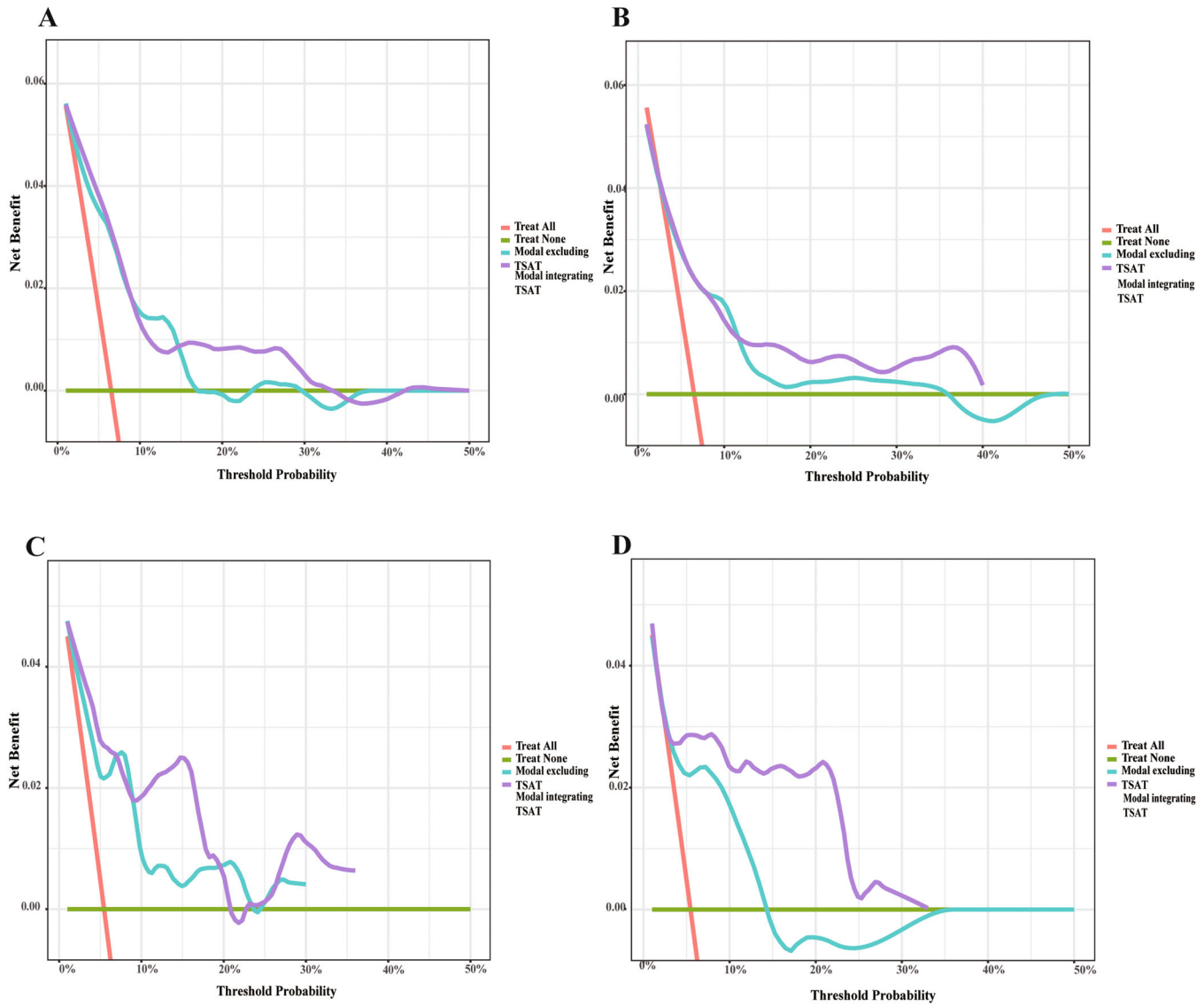

FIGURE 5 | Decision curve analysis for the model with addition of TSAT grade or excluding the TSAT grade. (A, B) Clinical application performance of BK viremia and BK nephropathy nomogram models in the training cohort respectively. (C, D) Clinical application performance of BK viremia and BK nephropathy nomogram models in the texting cohort respectively. The *y*-axis measures the net benefit. The blue line represents the model excluding the TSAT. The purple line represents the model with addition of TSAT grade. The orange line represents the assumption that all patients have BKPyV viremia or BKPyVAN. The green line represents the assumption that no patients have BKPyV viremia or BKPyVAN. The net benefit was calculated by subtracting the proportion of all patients who are false positive from the proportion who are true positive, weighting by the relative harm of forgoing treatment compared with the negative consequences of an unnecessary treatment. Here, the relative harm was calculated by$(\frac{p_t}{1-p_t})$, "p_t" (threshold probability) is where the expected benefit of treatment is equal to the expected benefit of avoiding treatment; at which time a patient will opt for treatment informs us of how a patient weighs the relative harms of false-positive results and false-negative results ($[a-c]/[b-d] = [1-p_t]/p_t$); $a-c$ is the harm from a false-negative result; $b-d$ is the harm from a false positive result. a, b, c, and d give, respectively, the value of true positive, false positive, false negative, and true negative.

Author Contributions

Y.Y., Z.W., and Y.F. contributed study design and paper writing. Y.Q., and Y.Q. contributed project oversight and paper revisiting. F.C., and Y.F. contributed data analysis and visualization. D.Z., G.F., and W.S. contributed study design and paper revisiting. All authors approved this manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.