Infection and Drug Resistance

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Metagenomic Next-Generation Sequencing-Assisted Risk Prediction and Stratification of Infections After Kidney Transplantation: A Case Study of COVID-19

Xin Ye[1,*], Chao Li[1,*], Zheng Zhou[1], Jiangnan Yang[2], Hao Jiang[1], Linkun Hu[1], Hao Pan[1], Xuedong Wei[1], Yuhua Huang[1], Yuxin Lin[1,3,*], Liangliang Wang[1,*]

[1]Department of Urology, The First Affiliated Hospital of Soochow University, Suzhou, 215000, People's Republic of China; [2]Department of Medicine, Dinfectome Inc., Nanjing, Jiangsu, 210000, People's Republic of China; [3]Center for Systems Biology, Soochow University, Suzhou, 215123, People's Republic of China

*These authors contributed equally to this work

Correspondence: Liangliang Wang; Yuxin Lin, Department of Urology, The First Affiliated Hospital of Soochow University, Suzhou, 215000, People's Republic of China, Tel +86-18061922022; +86-18862112682, Email wll234@163.com; linyuxin@suda.edu.cn

Background: During the COVID-19 pandemic, COVID-19 infection has severely damaged the transplanted kidney function and health of kidney transplant patients. This study aims to investigate the clinical characteristics, risk factors and predictors of severe COVID-19 in patients after kidney transplantation.

Material and Methods: The clinical data of patients with COVID-19 after kidney transplantation were collected from December 2022 to January 2023 at the First Affiliated Hospital of Soochow University. Logistic regression analysis was performed to identify risk factors for severe disease and to construct a nomogram model. Concurrently, metagenomic next-generation sequencing (mNGS) was employed to detect the sputum microbiome.

Results: A total of 58 patients were enrolled and were categorized into the common group (n=35) and the severe group (n=23) based on infection severity. The common group comprised 23 males with a mean age of 45.60 ± 9.11 years, while the severe group included 16 males with a mean age of 48.22 ± 9.95 years. Multivariate logistic analysis revealed that days of fever before hospitalization, C-reactive protein (CRP) and interleukin-10 (IL-10) on admission were significantly independent risk factors for severity, with an area under the ROC curve at 0.906. Comparison of the sputum microbiome revealed that there were no significant differences in α and β diversity between the two groups. *Streptococcus parasanguinis* was significantly more abundant in the specimens from the severe group, while *Gemella sanguinis* and *Gemella haemolysans* were significantly more abundant in the common group.

Conclusion: The severity of COVID-19 in kidney transplant patients is associated with days of fever before hospitalization, and the levels of CRP and IL-10 at admission, which also alter the abundance of certain species in the sputum microbiome. Therefore, it is necessary to actively monitor the clinical indicators of kidney transplant patients admitted with COVID-19 to reduce the risk of progression to severe disease.

Keywords: kidney transplantation, risk factors, CRP, IL-10, prediction model

Introduction

Kidney transplantation is an effective treatment for patients with end-stage kidney disease. However, the use of immunosuppressive drugs increases the susceptibility of these individuals to infections, which can potentially be life-threatening in severe cases.[1–4] During the COVID-19 pandemic, severe infections pose challenges to both the functioning of transplanted kidneys and the overall health of kidney transplant recipients.[5,6] Kidney transplant recipients exhibit significant high-risk characteristics in COVID-19 infection. Epidemiological data show higher

Received: 11 March 2025
Accepted: 18 August 2025
Published: 5 September 2025

rates of infection (12.3 per 1000 person-years), severe disease (48%), and short-term mortality (18% at 30 days) among this population. Meta-analysis of multicenter studies reveals a significantly elevated standardized mortality ratio (SMR) of 4.2 (95% CI 3.8–4.6).[7–9] Although some studies have summarized the clinical characteristics of COVID-19 among kidney transplant recipients,[10] there is a lack of comprehensive research on the risk factors and predictive clinical markers for severe COVID-19 post kidney transplantation.[11] This study analyzed the clinical characteristics and the composition of the sputum microbiome, identifying key risk indicators for severe infection. Based on these findings, a computational model was constructed to predict the risk of progression to severe disease in kidney transplant patients. The model could aid in earlier diagnosis, risk categorization, and personalized treatment of COVID-19 infections in these patients.

Materials and Methods

General Information

This study included a total of 58 kidney transplant inpatients at the Kidney Transplantation Center, Department of Urology, First Affiliated Hospital of Soochow University, from December 2022 to January 2023, who were diagnosed with the novel coronavirus through real-time RT-PCR of nasal or throat swabs and/or sputum metagenomic next-generation sequencing (mNGS). According to the Diagnosis and Treatment Protocol for COVID-19 (Trial Version 10), clinical classification was divided into four types: (1) mild, (2) moderate, (3) severe, and (4) critical. In this study, patients with mild and moderate conditions were included in the common group, while those with severe and critical conditions were included in the severe group. This study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University (number 2023–316) and was conducted according to the principles of the Helsinki Declaration. The Ethics Committee waived the need for informed consent.

Inclusion and Exclusion Criteria

The inclusion criteria were: (1) kidney transplant recipients aged 18–65 years; (2) patients diagnosed with COVID-19. Exclusion criteria were: (1) unavailable samples; (2) age < 18 years; (3) HIV infection; (4) suboptimal specimens or incomplete clinical data; and (5) concomitant malignant tumors or chronic obstructive pulmonary disease (COPD).

mNGS Sequencing

Nucleic Acid Extraction

Sputum samples were mixed with diluent in a centrifuge tube in a specified ratio, then proteinase K is added and mixed thoroughly. The mixture was incubated in a metal bath until the solution became clear or until there was no resistance when using a pipette gun. The liquefied sample was centrifuged at 1000 g for 5 minutes, and the supernatant was discarded. If no visible precipitate was present after centrifugation, 200 μL of the supernatant was retained for host removal. DNA and RNA were extracted using the TIANamp Micro DNA Kit and a proprietary viral RNA nucleic acid extraction kit, respectively. The quality of the extracted nucleic acids was evaluated using a NanoDrop, and the nucleic acids were quantified before library construction.

Library Construction and Sequencing

The extracted DNA was sonicated to create fragments of 150–300 bp. The sonication parameters were 30 seconds on and 30 seconds off, repeated for 10 cycles (Bioruptor Plus, Diagenode). The DNA library was prepared using the Hieff NGS C130P2 OnePot II DNA Library Prep Kit for MGI. The rRNA was removed from the total RNA, and the sample was purified using magnetic beads. The sample was then placed in a PCR machine and heated to 85°C for 7 minutes to fragment the RNA, followed by RNA library preparation. The library quality was controlled using an Agilent 2100, and the library concentration was quantified using a Qubit. The nucleic acid library was sequenced on the MGISEQ-200 instrument using 50 bp single-end sequencing.

Bioinformatics Analysis and Species Identification

First, the raw data obtained from sequencing was filtered using fastp (version 0.32.0) to remove low-quality sequences, contamination caused by adapters, duplicates, and sequences shorter than 36 bp. Next, the high-quality sequences were aligned to the human genome sequence (hs37d5) using bowtie2 (version 2.2.6). Sequences that did not match hs37d5 were retained and compared against an in-house pathogen database (containing over 20,000 species of bacteria, fungi, viruses, and parasites) to identify pathogens. Additionally, microbial classification was performed using Kraken (version 2.0.7), and species abundance was estimated using Bracken (version 2.5.0).

Statistical Methods

Continuous variables that follow a normal distribution were presented as the means ± standard deviations, while those that do not follow a normal distribution were represented as median and interquartile range. Categorical variables were reported as frequencies. Group comparisons were conducted using *t* tests or Mann–Whitney *U*-tests for continuous variables and $\chi 2$ tests or Fisher's exact tests for categorical variables. Univariate and multivariate analyses were performed using binary logistic regression models. Indicators with statistically significant differences in the univariate analysis were included in the multivariate binary logistic regression analysis to identify independent risk factors for severe COVID-19 in kidney transplant patients. The data were analyzed using SPSS version 27.0, and the p-value was calculated using the two-tailed method. A risk prediction nomogram model was constructed through multivariate logistic regression analysis, and the predictive probability value of each patient in this study was calculated and plotted on an ROC curve.[12]

Microbiome Analysis

The analysis of the sputum microbiome was conducted using R (version 4.0.1). Microbial alpha diversity was assessed by calculating the Shannon index, Simpson index, Ace index, and Chao1 index, and differences between groups were compared using the Wilcoxon rank-sum test. Beta diversity was evaluated by calculating the Bray-Curtis distance, and visualized using Principal Component Analysis (PCA) and Principal Coordinates Analysis (PCoA). Statistical differences between groups were assessed using multivariate analysis of variance (PERMANOVA, ADONIS). The Kruskal–Wallis rank-sum test was used to identify differences in microbial abundance at the genus and species levels between groups, with filtering criteria set at relative abundance > 1% and prevalence > 10%. The Spearman correlation between clinical characteristics and species-level relative abundance was calculated using the R package "cort.test". Linear Discriminant Analysis Effect Size (LEfSe) was employed to evaluate whether differences in microbial relative abundance between groups were statistically significant.

Results

Baseline Characteristics of the Patients

A total of 58 hospitalized patients with COVID-19 infection following kidney transplantation were enrolled in this study. Based on the severity of their illnesses, the patients were categorized into two groups: the common group (n=35, 60.34%) and the severe group (n=23, 39.66%). There were no significant differences between the two groups in terms of age, gender, days since transplant, history of chronic disease, incidence of pneumonia or rejection (P>0.05). The primary immunosuppressive regimen utilized in both groups consisted of calcineurin inhibitor (CNI), mycophenolate mofetil (MMF)/mycophenolate sodium enteric-coated tablets (EC-MPS) and glucocorticoid (Pre), with some patients additionally receiving rapamycin. No statistically significant differences were found in the immunosuppressive therapies administered to the two groups (P>0.05) (Table 1).

Clinical Presentations and Laboratory Tests

Fever was the primary clinical manifestation in these patients. The severe group exhibited a significantly greater number of fever days both before and during admission compared to the common group (P<0.05). At admission, the severe group had higher levels of creatinine, C-reactive protein (CRP), procalcitonin (PCT), IL-6 (interleukin-6), IL-10 (interleukin-

Table 1 Baseline Characteristics of the Patients

Characteristics	Common Type (N=35)	Severe Type (N=23)	P Value
Age (years)	45.60±9.11	48.22±9.95	0.307
Gender (male)	26(74.29)	16(69.57)	0.694
Days since transplant	36.00(4.00–67.00)	52.00(39.00–84.00)	0.161
Temperature peak	38.50(38.0–39.0)	39.00(38.60–39.00)	0.048
History of chronic disease			
No	4(11.43)	2(8.70)	1.000
Hypertension	29(82.86)	21(91.39)	0.601
Diabetes	10(28.57)	10(43.48)	0.243
Hepatitis B	9(25.71)	2(8.70)	0.202
History of pneumonia	2(5.71)	3(13.04)	0.621
History of rejection	6(17.14)	4(17.39)	1.000
Use of immunosuppressive agents			
Tacrolimus	26(74.29)	17(73.91)	0.975
Cyclosporin	9(25.71)	5(21.74)	0.729
EC-MPS	21(60.00)	10(43.48)	0.217
MMF	14(40.00)	12(52.17)	0.362
Prednisone	33(94.29)	22(95.65)	1.000
Medora	2(5.71)	1(4.35)	1.000
Rapamycin	3(8.57)	4(17.39)	0.551

10), IFN-γ, and D-dimer, as well as lower levels of albumin, absolute CD3+CD8+ values, and absolute CD3+CD4+ values compared to the common group (P<0.05) (Table 2).

Laboratory test results for these patients three months prior to infection were also collected (Table 3). In both the common and severe groups, there were significant changes in lymphocytes, hemoglobin, platelets, and albumin before and after infection (P<0.05). Specifically, there was a significant change in white blood cells (WBC) in the common group, and a statistically significant difference in creatinine in the severe group (P<0.05).

Table 2 Fever Status and Laboratory Tests Upon Admission of Patients in Both Groups

Characteristics	Common Type (N=35)	Severe Type (N=23)	P Value
Days of fever before hospitalization (days)	5.00(3.00–7.00)	7.00(3.00–14.00)	0.047
Days of fever during hospitalization (days)	1.00(0.00–3.00)	4.00(2.00–8.00)	0.001
Creatinine (umol/L)	119.80(94.30–172.50)	171.70(141.80–282.90)	0.005
Lymphocytes (10^9/L)	0.55(0.38–0.84)	0.40(0.28–0.84)	0.280
WBC (10^9/L)	4.20(3.33–6.96)	6.11(4.07–7.53)	0.065
Hemoglobin (g/L)	110.00(93.00–129.00)	100.00(88.00–135.00)	0.628
PLT (10^9/L)	173.00(134.00–217.00)	157.00(123.00–199.00)	0.485
CRP (mg/L)	11.43(4.47–19.99)	35.86(12.28–77.62)	0.006
PCT (ng/mL)	0.07(0.04–0.19)	0.19(0.09–0.60)	0.006
Albumin (g/L)	34.20(31.50–36.50)	30.40(27.60–34.10)	0.002
D-dimer (mg/L)	0.69(0.33–1.54)	1.56(0.76–2.40)	0.002
IL-6 (pg/mL)	9.20(3.90–21.50)	34.40(12.80–202.80)	<0.001
IL-10 (pg/mL)	2.20(0.00–4.60)	7.90(3.00–10.60)	<0.001
IFN-γ (pg/mL)	10.00(6.10–16.00)	14.50(8.90–24.60)	0.037
$CD4^+/CD8^+$ (%)	0.89(0.59–1.15)	0.69(0.51–1.50)	0.987
Absolute $CD3^+CD4^+$ count (cells/μL)	162.00(86.00–259.00)	95.00(59.00–142.00)	0.012
Absolute $CD3^+CD8^+$ count (cells/μL)	206.00(93.00–298.00)	101.00(74.00–205.00)	0.007

Table 3 Comparison of Laboratory Test Results Between the Two Groups Pre- and Post-Infection

Characteristics	Common Type (N=35)	P Value[a]	Severe Type (N=23)	P Value[b]	P Value[c]
Preinfection creatinine (μmol/L)	122.30 (99.80–145.90)	0.245	152.60 (107.60–189.10)	<0.001	0.064
Creatinine on admission (μmol/L)	119.80(94.30–172.50)		171.70(141.80–282.90)		0.005
Preinfection lymphocytes (10^9/L)	1.67(1.13–2.24)	<0.001	1.33(1.16–1.90)	<0.001	0.422
Lymphocytes on admission (10^9/L)	0.55(0.38–0.84)		0.40(0.28–0.84)		0.276
Preinfection WBC (10^9/L)	7.42(5.35–8.61)	<0.001	6.53(5.44–8.54)	0.100	0.886
WBC on admission (10^9/L)	4.20(3.33–6.96)		6.11(4.07–7.53)		0.065
Preinfection hemoglobin (g/L)	128.00(109.00–143.00	<0.001	115.00(100.00–137.00)	0.003	0.336
Hemoglobin on admission (g/L)	110.00(93.00–129.00)		100.00(88.00–135.00)		0.628
Preinfection PLT (10^9/L)	191.00(167.00–256.00)	0.012	204.00(174.00–225.00)	0.026	0.880
PLT on admission (10^9/L)	173.00(134.00–217.00)		157.00(123.00–199.00)		0.479
Preinfection albumin (g/L)	43.10(40.50–45.90)	<0.001	41.10(38.70–45.90)	<0.001	0.361
Albumin on admission (g/L)	34.20(31.50–36.50)		30.40(27.60–34.10)		0.002

Notes: [a]The P values of changes in laboratory indicators of common-type patients before and after infection; [b]The P values of changes in laboratory indicators of severe type patients before and after infection; [c]The P values of changes in laboratory indicators of common and severe patients.

Hospitalization Treatment Plans and Prognosis

Upon the diagnosis of COVID-19, the dosage of immunosuppressive agents was either reduced or discontinued. Patients were then treated with nematavir/ritonavir, methylprednisolone or dexamethasone, and low molecular weight heparin. The relevant laboratory results at admission, at discharge, and 3 months post discharge were compared and analyzed (Table 4). Compared to the admission values, the creatinine levels as well as the CRP and PCT levels significantly decreased at discharge in both groups. Lymphocytes WBCs, and PLTs significantly increased (P<0.05). The creatinine levels in the severe group exhibited a more pronounced decrease but remained slightly greater than the pre-infection. The maximum LDH concentration during treatment surpassed that before infection in both groups (P<0.001) but significantly decreased after treatment. The severe group experienced a more substantial increase in LDH compared to the common group (P<0.001).

Table 4 Laboratory Tests of the Two Groups Pre- and Post-Treatment

Characteristics	Common Type (N=35)	P Value[a]	Severe Type (N=23)	P Value[b]	P Value[c]
Creatinine on admission (μmol/L)	119.80(94.30–172.50)		171.70(141.80–282.90)		0.005
Creatinine at discharge (μmol/L)	106.70(82.20–142.40)	<0.001	138.30(110.30–210.70)	<0.001	0.017
Creatinine at 3 months postdischarge (μmol/L)	119.80(94.40–152.50)	0.156	152.70(107.70–210.70)	0.073	0.033
Lymphocytes on admission (10^9/L)	0.55(0.38–0.84)		0.40(0.28–0.84)		0.276
Lymphocytes at discharge (10^9/L)	0.80(0.48–1.20)	0.035	0.85(0.60–1.24)	0.002	0.861
WBC on admission (10^9/L)	4.20(3.33–6.96)		6.11(4.07–7.53)		0.065
WBC cells at discharge (10^9/L)	9.58(7.27–13.14)	<0.001	9.28(6.96–13.04)	0.002	0.639
Hemoglobin on admission (g/L)	110.00(93.00–129.00)		100.00(88.00–135.00)		0.628
Hemoglobin at discharge (g/L)	113.00(98.00–122.00)	0.316	95.00(83.00–119.00)	0.117	0.072
PLT on admission (10^9/L)	173.00(134.00–217.00)		157.00(123.00–199.00)		0.479
PLT at discharge (10^9/L)	217.00(154.00–253.00)	<0.001	196.00(137.00–213.00)	0.108	0.206
CRP on admission (mg/L)	11.43(4.47–19.99)		35.86(12.28–77.62)		0.006
CRP at discharge (mg/L)	2.19(1.08–3.87)	<0.001	4.98(1.38–14.33)	0.001	0.028
PCT on admission (ng/mL)	0.07(0.04–0.19)		0.19(0.09–0.60)		0.006
PCT at discharge (ng/mL)	0.05(0.03–0.10	0.028	0.09(0.04–0.32)	0.042	0.048
Preinfection LDH (U/L)	185.70(172.30–215.40)		215.30(186.80–234.30)		0.059
LDH MAX during treatment (U/L)	249.30(216.00–316.80)	<0.001	384.00(289.20–618.20)	<0.001	<0.001
LDH MIN during treatment (U/L)	199.80(177.30–239.00)	0.095	248.90(191.10–288.70)	0.073	0.039

Notes: [a]The P values of changes in laboratory indicators of common-type patients before and after treatment; [b]The P values of changes in laboratory indicators of severe type patients before and after treatment; [c]The P values of changes in laboratory indicators of common and severe patients.

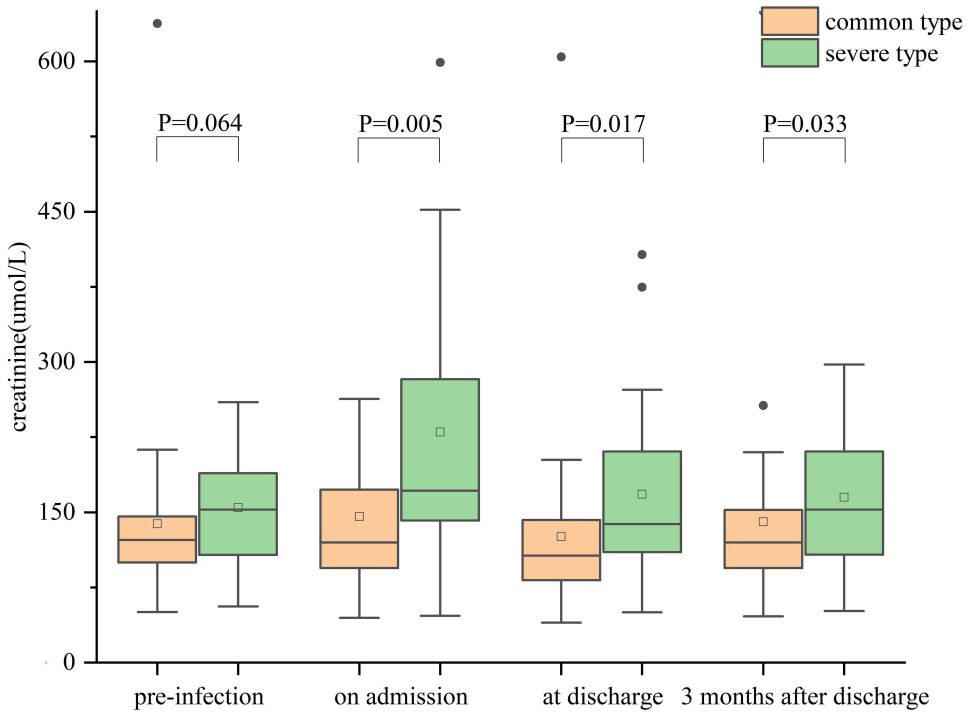

Figure 1 The variations in creatinine levels between the two groups during the entire treatment process.

Two patients experienced acute rejection during their hospital stay, both of whom had undergone transplantation within one month prior to the event. Two patients experienced acute kidney failure and subsequently recovered after treatment. Except for three deaths in the severe group, all other patients were discharged successfully. After three months of follow-up, lung CT scans and kidney function tests revealed complete absorption of lung lesions in the common group, while some patients in the severe group still exhibited residual lesions. During the entire treatment process, the creatinine levels in the severe group were significantly greater than those in the common group (Figure 1).

Analysis of Risk Factors for Progression to Severe Disease

Univariate analysis results indicate that length of hospital stay, number of days with fever before hospitalization, and clinical indicators on the day of admission (IL-6, IL-10, absolute values of CD3+CD4+, absolute values of CD3+CD8+, creatinine, albumin, CRP) were all associated with the progression to severe illness (P<0.05, Table 5). Specifically, days

Table 5 Univariate Analysis of Risk Factors for Progression to Severe Disease

Indicators	Coefficient of Regression	Standard Error	Value of Wald	Degree of Freedom	P-Value	OR	OR 95% CI
Length of hospital stay	0.136	0.051	7.137	1	0.008	1.146	1.037–1.267
Days of fever before hospitalization	0.129	0.060	4.543	1	0.033	1.137	1.010–1.280
IL-6 on admission	0.020	0.008	5.745	1	0.017	1.020	1.004–1.036
IL-10 on admission	0.444	0.121	13.463	1	<0.001	1.560	1.230–1.977
Absolute $CD3^+CD4^+$ count on admission	−0.009	0.004	5.701	1	0.017	0.991	0.984–0.998
Absolute $CD3^+CD8^+$ count on admission	−0.008	0.003	5.648	1	0.017	0.993	0.986–0.999
Creatinine on admission	0.006	0.003	3.891	1	0.049	1.006	1.000–1.011
Albumin on admission	−0.240	0.086	7.831	1	0.005	0.787	0.665–0.931
LDH MAX on admission	0.007	0.002	8.895	1	0.003	1.007	1.002–1.012
LDH MIN on admission	0.009	0.004	4.606	1	0.032	1.009	1.001–1.017
Albumin on admission	−0.240	0.086	7.831	1	0.005	0.787	0.665–0.931
CRP on admission	0.033	0.012	7.816	1	0.005	1.033	1.010–1.057

Table 6 Multivariate Analysis of Risk Factors for Progression to Severe Disease

Indicators	Coefficient of Regression	Standard Error	Value of Wald	Degree of Freedom	P-Value	OR	OR 95% CI
Days of fever before admission	0.206	0.102	4.117	1	0.042	1.229	1.007–1.499
IL-10 on admission	0.564	0.162	12.090	1	<0.001	1.758	1.279–2.416
CRP on admission	0.028	0.016	2.998	1	0.083	1.028	0.996–1.061
Constant	−5.152	1.384	13.859	1	<0.001	0.006	

of fever before hospitalization, IL-10 and CRP on admission were identified as independent risk factors. (P<0.1, Table 6). The area under the ROC curve (AUC = 0.906) and the Hosmer-Lemeshow test (χ^2 = 6.001, P = 0.647) indicated that the model performed well (Figure 2A). Based on the results of the multivariate logistic regression analysis, a nomogram was developed to visually represent the predictive model (Figure 2B).

Characteristics of the Sputum Microbiome

In the study, 16 sputum samples were collected and subjected to mNGS, with 6 from the common group and 10 from the severe group. α diversity analysis showed no statistically significant differences in the Ace, Chao, Shannon, and Simpson indices between the common and severe groups (P>0.05, Figure 3A–D). In β diversity analysis, both PCA and PCoA analyses revealed that the samples from the two groups could not be significantly separated (p > 0.05, Figure 3E and F), indicating that the severity of COVID-19 infection in kidney transplant patients did not significantly alter the lung microbiome. However, there was a difference in the Bray-Curtis distance between the two groups, suggesting higher heterogeneity within the severe group (Figure 3G).

The sputum microbiome composition was similar between the common and severe groups, with the highest proportions of the *Rothia* and *Streptococcus*. At the species level, *Gemella sanguinis* and *Gemella haemolysans* showed significantly lower relative abundance in severe group, while *Streptococcus parasanguinis* had significantly lower relative abundance in common group (Figure 4A). At the genus level, the Gemella genus also exhibited significantly lower relative abundance in severe group (Figure 4B).

LEfSe analysis was used to identify the potential biomarkers in the two groups. Using criteria of LDA scores≥2 and P<0.05, six discriminative biomarkers were identified at the species level. *Streptococcus parasanguinis* was significantly enriched in the severe group, while *Gemella sanguinis, Gemella haemolysans, Prevotella multiformis, Oribacterium sinus*, and *Eubacterium nodatum* were significantly enriched in the common group. At the genus level, *Gemella* and *Oribacterium* were also significantly enriched in the common group (Figure 4C).

In severe group, the significantly reduced *Gemella sanguinis* and *Gemella haemolysans* were negatively correlated with peak body temperature and IL-10, with *Gemella haemolysans* also showing a significant negative correlation with CRP. In contrast, *Streptococcus parasanguinis*, which was significantly enriched in severe group, was positively correlated with peak body temperature, IL-6, and IL-10. These findings suggest different roles of various bacteria in severe COVID-19 in kidney transplant patients, where *Gemella sanguinis* and *Gemella haemolysans* may be associated with anti-inflammatory effects, while *Streptococcus parasanguinis* may be linked to pro-inflammatory effects (Figure 4D).

Discussion

Due to long-term used of immunosuppressants, kidney transplant recipients have weakened immune systems. This makes them more vulnerable to COVID-19 infection and prone to experiencing more severe symptoms than the general population. The mortality rate among this cohort during the COVID-19 pandemic has been approximately 30%.[13–15] Although there has been a notable decline in recent years, the mortality rate from infections in these patients remains considerably greater than that of the general population.[16] Effectively reducing COVID-19 mortality depends crucially on

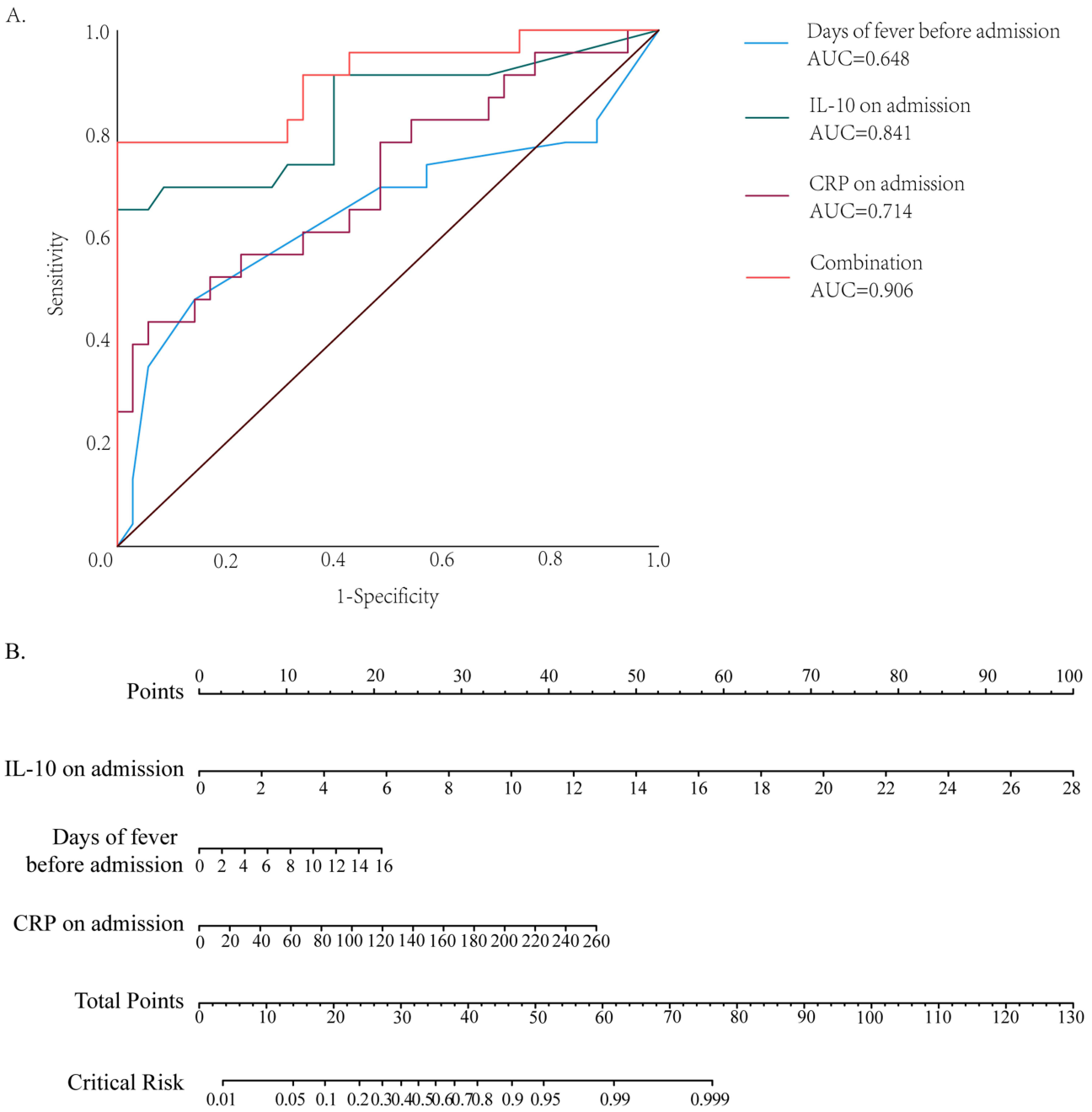

Figure 2 Risk prediction and assessment for severe COVID-19 post-kidney transplantation (**A**) The ROC curve of independent risk factors for the progression of COVID-19 to severe disease after kidney transplantation. (**B**) Nomogram for predicting the risk of severe COVID-19 after kidney transplantation.

preventing severe disease progression. Hence, this study summarized the clinical characteristics and sputum microbiome features of mild and severe patients to identify risk factors for the progression of severe illness.

Fever is a common symptom among individuals infected with COVID-19. In our study, every patient experienced fever, although the intensity and duration varied from person to person. However, the relationship between fever and the severity of COVID-19 infection remains underexplored. Notably, our findings suggest that patients with severe COVID-19 often experience a fever lasting longer than one week prior to hospital admission. It is worth mentioning that there appears to be no significant correlation between the temperature peak reached and the severity of the disease. Due to individual differences and the use of immunosuppressants among solid organ transplant recipients, early symptoms may be obscured, resulting in low-grade fevers even in severe cases.[17] Multivariate regression analysis in this study indicated

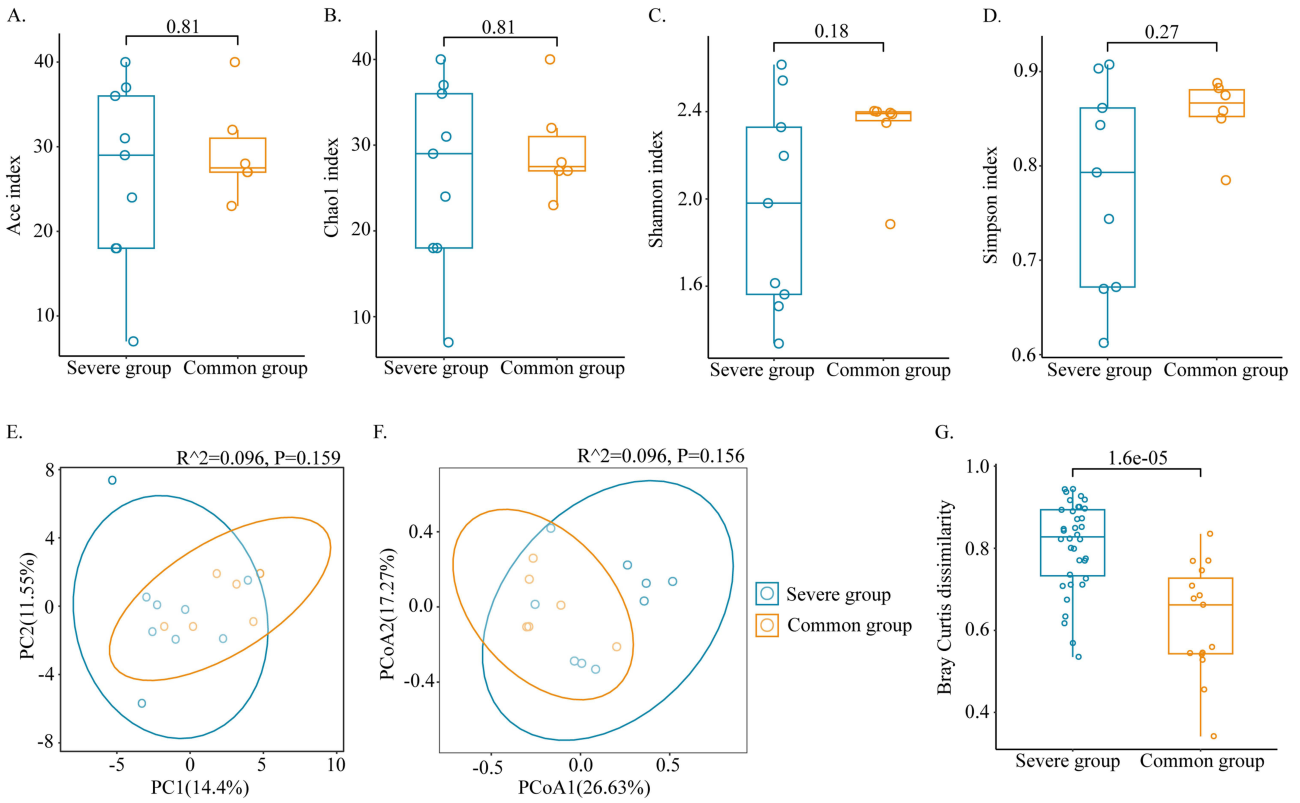

Figure 3 Comparison of sputum microbiome diversity between the common and severe Groups. (**A**) Ace index. (**B**) Chao1 index. (**C**) Shannon index. (**D**) Simpson index. (**E**) PCA analysis. (**F**) PCoA analysis. (**G**) Bray–Curtis distance between samples.

that the duration of persistent fever prior to admission is an independent risk factor for disease progression to a severe stage. Therefore, increased vigilance is essential for timely assessment and effective management of patients who present with persistent fevers.

COVID-19 often triggers an inflammatory response, leading to the release of diverse inflammatory factors, such as IL-6, IL-10, IFN-γ, and CRP, which is particularly evident in severe cases.[18,19] Bivona et al established a connection between the concentrations of these inflammatory markers and the severity of COVID-19, highlighting a notable increase in IL-6 levels in severe patients.[20] Similarly, Zeng et al recognized PCT, CRP, and IL-6 as indicators of disease severity and mortality risk in COVID-19 patients.[21] Our research revealed significantly elevated levels of CRP, PCT, IL-6, IL-10, and IFN-γ in the severe group compared to the common group upon admission. Interestingly, contrary to prevailing reports, our findings indicate that the ability of IL-10 to predict severe illness progression might surpass that of IL-6. Carlini et al posited that IL-10, as an endogenous danger signal, functions as a prognostic marker for disease severity and mortality in patients infected with the novel coronavirus.[22] Weiss-Tessbach et al further underscored the significance of IL-10 in differentiating between severe and critical COVID-19 patients.[23]

Multivariate analysis from our study demonstrated that admission levels of IL-10 and CRP, along with the duration of fever before admission, were independent risk factors ($P<0.1$). Based on the duration of fever before admission as well as the CRP and IL-10 levels at admission, a nomogram model was constructed to predict the risk of disease progression towards a severe stage. The corresponding AUC was calculated to be 0.906, suggesting excellent predictive ability for these three factors and their respective models.

COVID-19 frequently leads to acute kidney injury (AKI), which affects approximately ~20–40% of severe patients.[24,25] AKI is considered a marker of disease severity and an unfavorable prognostic factor for survival.[26,27] The kidney function assessment in this study revealed that the common group did not exhibit an increase in creatinine levels postinfection; in fact, there was even a transient decrease during treatment, possibly attributed to the reduction or discontinuation of CNI drugs. Conversely, the severe group experienced a significant increase in creatinine levels after

https://doi.org/10.2147/IDR.S521111

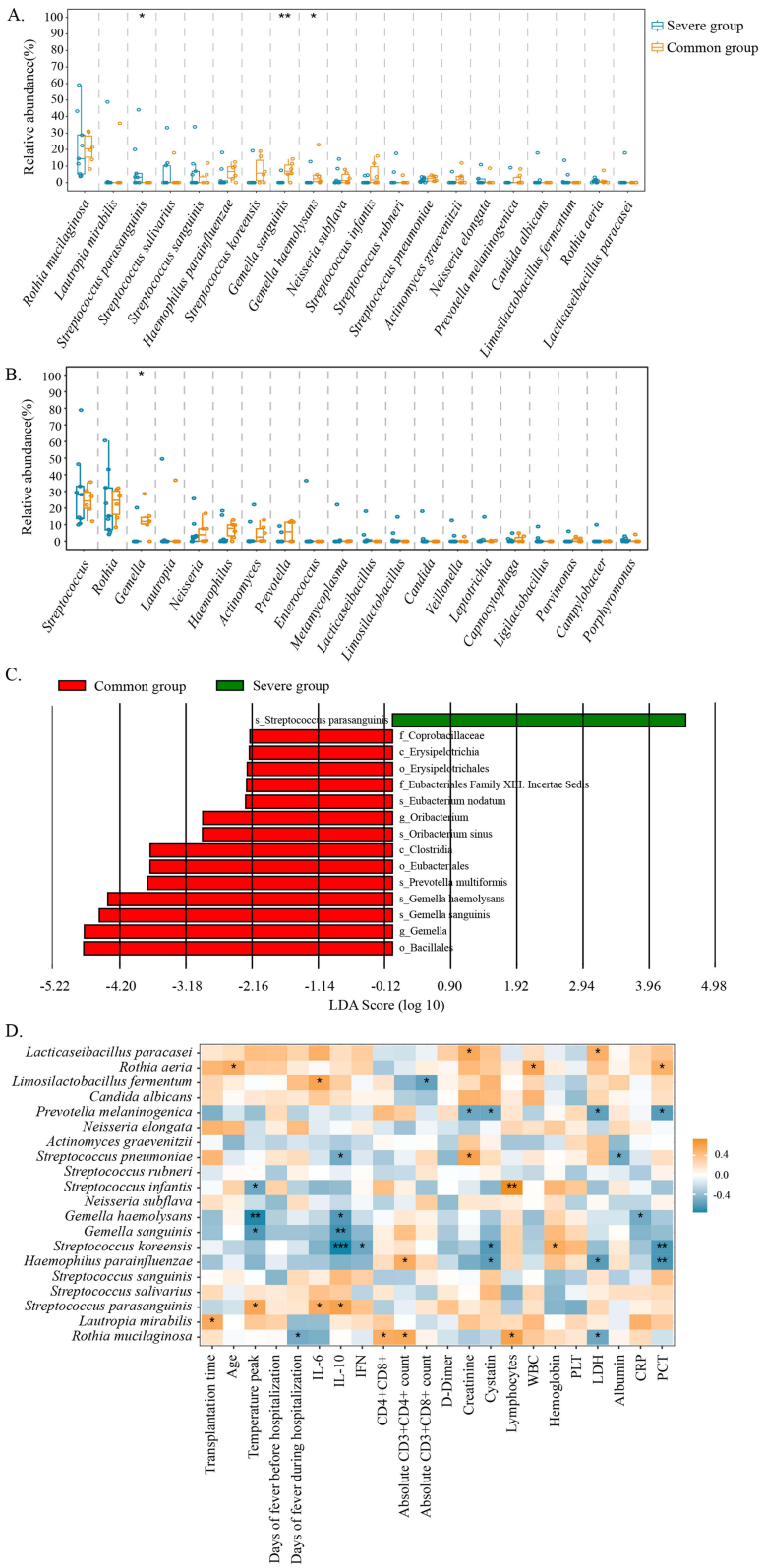

Figure 4 Differential analysis of the top 20 species between the common and severe group and correlation with clinical indicators. (**A**) Analysis of relative abundance differences at the species level. (**B**) Analysis of relative abundance differences at the genus level. (**C**) The bar plots represent the significantly differential taxa between groups. (**D**) Correlation analysis between relative abundance of species and clinical indicators.
Note: *p<0.05; **p<0.01; ***p<0.001.

infection, with two patients requiring continuous kidney replacement therapy. Kidney pathology confirmed acute tubular necrosis, consistent with previous reports.[28,29] Additionally, LDH levels were significantly elevated in the severe group, while some patients exhibited a ≥50% reduction in PLT compared to baseline levels along with decreased hemoglobin. Considering Jodele's proposed diagnostic criteria for transplantation-associated thrombotic microangiopathy (TA-TMA), it is plausible that severe COVID-19 infection may induce TA-TMA and impact kidney function.[30] However, further research is warranted to explore this hypothesis.

In this study, we discovered that the absolute CD3+CD4+ T-cell count could serve as a significant predictor of severe COVID-19 infection among kidney transplant recipients. Calvet et al conducted a prospective study that underscored the predictive value of lymphocyte subsets, specifically CD3+CD4+ T cells, in gauging the severity of COVID-19 infection.[31] The authors recommend determining lymphocyte subsets upon admission. Similarly, a retrospective study conducted by Liana et al revealed that CD4+ and CD8+ cell counts were notably lower in critically ill COVID-19 patients than in those with mild-to-moderate infection.[32] Nonetheless, the CD3+CD4+ cell count cannot be regarded as a standalone predictor of severe COVID-19 infection in kidney transplant recipients, and further validation through a larger sample size is warranted.

In this cohort of kidney transplant recipients with COVID-19, we collected 16 sputum samples for mNGS analysis. Consistent with the findings by Ye et al (2021–2023), which identified SARS-CoV-2 as the predominant pathogen in kidney transplant recipients, mNGSdemonstrated high diagnostic sensitivity for this virus.[33] Furthermore, mNGS showed significant advantages in detecting coinfections. This is supported by Huang et al, who reported superior diagnostic performance of mNGS over conventional methods for respiratory pathogen detection in bronchoalveolar lavage fluid from kidney transplant recipients.[34] Additionally, Tian et al revealed complex viral profiles in infected kidney transplant recipients using mNGS during the pandemic.[35] In this study, we conducted comparative analysis of microbial profiles between critically ill and common patient subgroups to identify taxa associated with disease severity.

In severe patients, *Streptococcus parasanguinis* was significantly enriched, while *Gemella sanguinis* and *Gemella haemolysans* were significantly reduced. *Streptococcus* is highly prevalent in the oral cavities of healthy individuals and has been shown to have a significant correlation with inflammatory markers in certain disease populations.[36–38] *Gemella* is a catalase-negative, facultatively anaerobic, Gram-positive coccus that exists in a symbiotic relationship with humans but can become an opportunistic pathogen. Among them, *Gemella haemolysans* is a commensal bacterium of the upper respiratory tract, predominantly found in the oral cavity, and can occasionally cause endocarditis and meningitis.[39,40] A study showed that the concentration of *Gemella haemolysans* was significantly higher in non-COVID-19 patients. Moreover, its concentration was positively correlated with chlorogenic acid methyl ester (CME) in the serum. This suggests that *Gemella* may have many beneficial properties for COVID-19.[41]

The limitations are also worthy of being concerned. In this study, univariate and multivariate regression analyses were utilized to select variables for model construction. It would be better to use rational clinical judgment and select all variables which might be potential predictors of outcomes and confounders. However, given the current sample size collected, we performed regression analyses before model construction in order to balance the number of variables and the sample size, and to prioritize key variables to avoid overfitting during model training. In our future work, large-sample and multi-center-based validations with incorporation of novel biomarkers and machine learning techniques would be conducted to convince and improve the robustness and generality of the proposed model.

Conclusions

In conclusion, the prognosis for COVID-19 patients who have undergone kidney transplantation is generally positive; however, severe cases still pose a risk of kidney damage and mortality. The duration of fever before admission, as well as the CRP and IL-10 levels at admission, emerged as independent risk factors for severe COVID-19 among kidney transplant recipients, demonstrating notable predictive value. Prompt treatment should be provided to kidney transplant patients diagnosed with COVID-19 to reduce the risk of progression to severe illness.

Abbreviations

CRP, C-reactive protein; IL, interleukin; IFN-γ, interferon-gamma; mNGS, metagenomic next-generation sequencing; WBC, white blood cell; PLT, platelet count; LDH, lactate dehydrogenase; PCT, procalcitonin; CNI, calcineurin inhibitor; MMF, mycophenolate mofetil; EC-MPS, mycophenolate sodium enteric-coated tablets; ROC, receiver operating characteristic; AUC, Area under the ROC Curve; AKI, acute kidney injury; TA-TMA, transplantation-associated thrombotic microangiopathy.

Data Sharing Statement

No datasets were generated or analyzed during the current study.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University (Approval No. [2023-316]), which waived the requirement for informed consent due to the retrospective nature of the study and the use of de-identified data. All patient data were anonymized and handled with strict confidentiality in compliance with institutional and international ethical standards. All kidneys were procured from either living related donors or deceased donors, with formal approval and written informed consent obtained from the donors or their legal representatives, in full compliance with the China Human Organ Donation and Transplantation Regulations. All kidneys were donated voluntarily. The organ procurement and transplantation procedures were conducted in strict accordance with the Declaration of Istanbul.

Acknowledgments

We sincerely thank Dinfectome, Inc., Nanjing, China, for providing help with the mNGS and interpretation of the results.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was supported by the National Natural Science Foundation of China (Grant Number: 82270787), the Suzhou Municipal Science and Technology Plan Project (Project number: SLJ2021002) and the Natural Science Foundation of Jiangsu Province (Grant number: BK20210094).

Disclosure

The authors declare no conflict of interest.

References

1. Sagnelli C, Sica A, Gallo M, et al. Renal involvement in COVID-19: focus on kidney transplant sector. *Infection*. 2021;49(6):1265–1275.
2. Marcen R. Immunosuppressive drugs in kidney transplantation: impact on patient survival, and incidence of cardiovascular disease, malignancy and infection. *Drugs*. 2009;69(16):2227–2243.
3. Soliman K, Calimlim IK, Perry A, et al. Evolving trends in immunosuppression use and cytomegalovirus infection risk over the past decade in kidney transplantation. *Transpl Infect Dis*. 2024;26(5):e14318. doi:10.1111/tid.14318
4. Weigang S, Fuchs J, Zimmer G, et al. Within-host evolution of SARS-CoV-2 in an immunosuppressed COVID-19 patient as a source of immune escape variants. *Nat Commun*. 2021;12(1):6405. doi:10.1038/s41467-021-26602-3
5. Telenti A, Arvin A, Corey L, et al. After the pandemic: perspectives on the future trajectory of COVID-19. *Nature*. 2021;596(7873):495–504. doi:10.1038/s41586-021-03792-w
6. Kinnunen S, Karhapaa P, Juutilainen A, Finne P, Helantera I. Secular trends in infection-related mortality after kidney transplantation. *Clin J Am Soc Nephrol*. 2018;13(5):755–762. doi:10.2215/CJN.11511017

7. Thaunat O, Legeai C, Anglicheau D, et al. IMPact of the COVID-19 epidemic on the moRTAlity of kidney transplant recipients and candidates in a French nationwide registry sTudy (IMPORTANT). *Kidney Int*. 2020;98(6):1568–1577. doi:10.1016/j.kint.2020.10.008
8. Mohan S, King KL, Husain SA, Schold JD. COVID-19–associated mortality among kidney transplant recipients and candidates in the United States. *Clin J Am Soc Nephrol*. 2021;16(11):1695–1703. doi:10.2215/CJN.02690221
9. Elias M, Pievani D, Randoux C, et al. COVID-19 infection in kidney transplant recipients: disease incidence and clinical outcomes. *J Am Soc Nephrol*. 2020;31(10):2413–2423. doi:10.1681/ASN.2020050639
10. Fava A, Cucchiari D, Montero N, et al. Clinical characteristics and risk factors for severe COVID-19 in hospitalized kidney transplant recipients: a multicentric cohort study. *Am J Transplant*. 2020;20(11):3030–3041. doi:10.1111/ajt.16246
11. Kremer D, Pieters TT, Verhaar MC, et al. A systematic review and meta-analysis of COVID-19 in kidney transplant recipients: lessons to be learned. *Am J Transplant*. 2021;21(12):3936–3945. doi:10.1111/ajt.16742
12. Park SY. Nomogram: an analogue tool to deliver digital knowledge. *J Thorac Cardiovasc Surg*. 2018;155(4):1793. doi:10.1016/j.jtcvs.2017.12.107
13. Cravedi P, Mothi SS, Azzi Y, et al. COVID-19 and kidney transplantation: results from the TANGO International Transplant Consortium. *Am J Transplant*. 2020;20(11):3140–3148. doi:10.1111/ajt.16185
14. Toapanta N, Torres IB, Sellares J, Chamoun B, Seron D, Moreso F. Kidney transplantation and COVID-19 renal and patient prognosis. *Clin Kidney J*. 2021;14(Suppl 1):i21–i29. doi:10.1093/ckj/sfab030
15. Akalin E, Azzi Y, Bartash R, et al. Covid-19 and kidney transplantation. *N Engl J Med*. 2020;382(25):2475–2477. doi:10.1056/NEJMc2011117
16. Pinchera B, Spirito L, Ferreri L, et al. SARS-CoV-2 in kidney transplant patients: a real-life experience. *Front Med*. 2022;9:864865. doi:10.3389/fmed.2022.864865
17. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. *Nat Rev Microbiol*. 2021;19(3):141–154.
18. Chen Z, Xu W, Ma W, et al. Clinical laboratory evaluation of COVID-19. *Clin Chim Acta*. 2021;519:172–182.
19. Chang SH, Minn D, Kim SW, Kim YK. Inflammatory markers and cytokines in moderate and critical cases of COVID-19. *Clin Lab*. 2021;67(9). doi:10.7754/Clin.Lab.2021.210142
20. Bivona G, Agnello L, Ciaccio M. Biomarkers for prognosis and treatment response in COVID-19 patients. *Ann Lab Med*. 2021;41(6):540–548. doi:10.3343/alm.2021.41.6.540
21. Zeng F, Huang Y, Guo Y, et al. Association of inflammatory markers with the severity of COVID-19: a meta-analysis. *Int J Infect Dis*. 2020;96:467–474.
22. Carlini V, Noonan DM, Abdalalem E, et al. The multifaceted nature of IL-10: regulation, role in immunological homeostasis and its relevance to cancer, COVID-19 and post-COVID conditions. *Front Immunol*. 2023;14(1161067).
23. Weiss-Tessbach M, Ratzinger F, Obermueller M, et al. Biomarkers for differentiation of coronavirus disease 2019 or extracorporeal membrane oxygenation related inflammation and bacterial/fungal infections in critically ill patients: a prospective observational study. *Front Med*. 2022;9:917606.
24. Richardson S, Hirsch JS, Narasimhan M, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City area. *JAMA*. 2020;323(20):2052–2059. doi:10.1001/jama.2020.6775
25. Moledina DG, Simonov M, Yamamoto Y, et al. The association of COVID-19 with acute kidney injury independent of severity of illness: a multicenter cohort study. *Am J Kidney Dis*. 2021;77(4):490–499e491. doi:10.1053/j.ajkd.2020.12.007
26. Hilton J, Boyer N, Nadim MK, Forni LG, Kellum JA. COVID-19 and acute kidney injury. *Crit Care Clin*. 2022;38(3):473–489.
27. Chan L, Chaudhary K, Saha A, et al. AKI in hospitalized patients with COVID-19. *J Am Soc Nephrol*. 2021;32(1):151–160. doi:10.1681/ASN.2020050615
28. Su H, Yang M, Wan C, et al. Renal histopathological analysis of 26 postmortem findings of patients with COVID-19 in China. *Kidney Int*. 2020;98(1):219–227. doi:10.1016/j.kint.2020.04.003
29. Diao B, Wang C, Wang R, et al. Human kidney is a target for novel severe acute respiratory syndrome coronavirus 2 infection. *Nat Commun*. 2021;12(1):2506. doi:10.1038/s41467-021-22781-1
30. Jodele S, Laskin BL, Dandoy CE, et al. A new paradigm: diagnosis and management of HSCT-associated thrombotic microangiopathy as multi-system endothelial injury. *Blood Rev*. 2015;29(3):191–204.
31. Calvet J, Gratacos J, Amengual MJ, et al. CD4 and CD8 lymphocyte counts as surrogate early markers for progression in SARS-CoV-2 pneumonia: a prospective study. *Viruses*. 2020;12(11):1277. doi:10.3390/v12111277
32. Liana P, Samosir AP, Sari NP, et al. CD4+ and CD8+ cell counts are significantly correlated with absolute lymphocyte count in hospitalized COVID-19 patients: a retrospective study. *PeerJ*. 2023;11:e15509. doi:10.7717/peerj.15509
33. Ye X, Lin Y, Yang J, et al. Deciphering the pathogen heterogeneity for precise diagnosis and personalized therapeutics of infections after kidney transplantation: insights from metagenomic next-generation sequencing. *Front Cell Infect Microbiol*. 2024;14(1456407). doi:10.3389/fcimb.2024.1456407
34. Huang Z, Hu B, Li J, et al. Metagenomic versus targeted next-generation sequencing for detection of microorganisms in bronchoalveolar lavage fluid among renal transplantation recipients. *Front Immunol*. 2024;15:1443057.
35. Tian X, Duan W, Zhang X, et al. Metagenomic next-generation sequencing reveals the profile of viral infections in kidney transplant recipients during the COVID-19 pandemic. *Front Public Health*. 2022;10:888064.
36. Lei Y, Li M, Zhang H, et al. Comparative analysis of the human microbiome from four different regions of China and machine learning-based geographical inference. *mSphere*. 2025;10(1):e0067224. doi:10.1128/msphere.00672-24
37. Wang X, Song X, Gao J, et al. Impact of oral flora in tongue coating and saliva on oral cancer risk and the regulatory role of Interleukin-8. *Cytokine*. 2025;185:156821. doi:10.1016/j.cyto.2024.156821
38. Lyv ZY, Wang LJ, Xu MX, Bai XF, Cao LJ. [Association between the structure of intestinal flora and inflammatory response in children with sepsis: a prospective cohort study]. *Zhongguo Dang Dai Er Ke Za Zhi*. 2024;26(6):567–574. doi:10.7499/j.issn.1008-8830.2312113 Xhosa
39. Lee MR, Lee SO, Kim SY, Yang SM, Seo YH, Cho YK. Brain abscess due to Gemella haemolysans. *J Clin Microbiol*. 2004;42(5):2338–2340.
40. Anil M, Ozkalay N, Helvaci M, et al. Meningitis due to Gemella haemolysans in a pediatric case. *J Clin Microbiol*. 2007;45(7):2337–2339. doi:10.1128/JCM.00208-07
41. Liu J, Liu S, Zhang Z, et al. Association between the nasopharyngeal microbiome and metabolome in patients with COVID-19. *Synth Syst Biotechnol*. 2021;6(3):135–143. doi:10.1016/j.synbio.2021.06.002