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Correlation between peripheral lymphocyte subsets monitoring and COVID-19 pneumonia in kidney transplant recipients

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Abstract

Objectives In kidney transplant recipients (KTRs), immune monitoring of peripheral lymphocyte subsets (PLS) reflects the real immune status and aids in the diagnosis of the occurrence and development of infectious diseases, including COVID-19. Exploring the PLS of COVID-19 pneumonia in KTRs is important.

Methods In this study, a total of 103 KTRs were divided into mild pneumonia (MP) and severe pneumonia (SP) groups, as well as a stable group. The clinical information and PLS data were assessed via *t* or Mann-Whitney test and receiver operating curve analysis. Logistic regression was employed to identify the risk factors, and Spearman's correlation analysis was used to identify correlations.

Results Lymphopenia is a common manifestation of COVID-19 in KTRs, and it is positively related to the severity of COVID-19 pneumonia. The CD3 + T-cell count had the highest AUC between the MP and the SP. Multiple PLS measures were found to be independent risk factors for COVID-19 pneumonia progression in KTRs.

Conclusions This study revealed a robust correlation between PLS and severe COVID-19 pneumonia progression in KTRs. PLS monitoring could facilitate real-time diagnosis and therapy for infection, as well as timely and precisive adjustment of immunosuppression instructions, for KTRs with COVID-19.

Keywords Kidney transplantation, COVID-19, Peripheral lymphocyte subsets, Severe pneumonia, Immune monitoring

Introduction

The corona virus disease 2019 (COVID-19) was initially identified and then spread worldwide rapidly since December 2019, and it has become the largest global public health challenge to date. According to the World Health Organization, by March 2023, the number of confirmed cases of COVID-19 had reached 759 million worldwide, and the number of deaths had exceeded 6.8 million, seriously threatening human safety. In the context of this epidemic, kidney transplant recipients (KTRs) are reported to have a 2–5 times higher incidence than the general population [1]. Furthermore, owing to the large population and long-term use of immunosuppressive drugs, KTRs tend to be at increased risk of developing severe to fatal COVID-19, concomitant acute kidney

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injury (AKI), and persistent post-COVID-19 symptoms [2, 3], with an unfavorable prognosis.

COVID-19 clearly causes serious damage to immune function, which can be aggravated by kidney-related diseases. Several studies have reported a notably greater incidence of thrombocytopenia and lymphopenia in KTRs, as well as in patients with end-stage renal disease (ESRD), than in those without underlying kidney disease [4]. Despite the rapid development of immunological knowledge about COVID-19, little is known about the immune status against the COVID-19 virus in immunocompromised populations, particularly in KTRs. More severe immune dysfunction may be related to long-term exposure to immunosuppression, which can predispose KTRs to severe forms of COVID-19 infection [5].

Monitoring the immune function of patients after COVID-19 infection can help with timely intervention and thus significantly affect the prognosis and survival of patients after kidney transplantation (KTx). Peripheral blood is still the most common sample source for immune status detection, despite an increasing number of immune biomarkers being widely used and improvements in detection technology in clinical practice. Analysis of peripheral lymphocyte subsets (PLSs) is an effective and minimally invasive approach to reflect the basic immune status of follow-up renal transplant patients [6]. Previous studies have investigated the correlation between PLS and pneumonia following KTx to assess its diagnostic utility for pneumonia other than COVID-19 [7, 8]. Therefore, our study better characterized and compared the fundamental immunological differences between stable and COVID-19 pneumonia patients after KTx to obtain data concerning COVID-19. Furthermore, this study aims to understand how to provide new insights into monitoring, individualized prevention, and therapy for adjusting immunosuppression in patients with severe COVID-19 pneumonia after KTx via PLS monitoring.

Methods

Patient recruitment and blood specimen collection

All KTRs and non-transplanted patients were enrolled from the Transplantation Center, The 3rd Xiangya Central South University, between December 15th, 2022, and March 31st, 2023. Another group of stable outpatient KTRs was also recruited as a control group during another period. Informed consent was obtained from each patient. In addition, the study adhered to the Helsinki Declaration and received approval from the Institutional Review Board of Third Xiangya Hospital, Central South University (IRB No.21176).

The kidney allografts were procured from donations after citizen death (DCD) or from close relatives. All kidney transplantation surgeries were achieved and authorized by the Ethics Committee of the Third Xiangya Hospital. The organ allocation scheme was left to the China Organ Transplant Response System's discretion. The maintenance immunosuppressants consisted of calcineurin inhibitors (CNIs), mycophenolate mofetil (MMF), and corticosteroids. Clinical manifestations, laboratory test results, and radiological examinations were employed to diagnose and describe the condition of COVID-19 pneumonia patients. Graft renal function was calculated via *the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Eq. (2009)* [9].

Confirmed cases of COVID-19 infection were diagnosed as positive nucleic acid test or antigen test using nasal or pharyngeal swab specimens. With respect to the severity of the disease, COVID-19 pneumonia patients were divided into mild pneumonia (MP) and severe pneumonia (SP) groups. The diagnostic criteria for MP included the following: (1) epidemiological history; (2) fever or other respiratory symptoms; (3) typical computed tomography (CT) image abnormities of viral pneumonia; (4) positive result of reverse transcription–polymerase chain reaction (RT–PCR) for SARS-CoV-2 ribonucleic acid, while SP additionally met at least one of the following conditions: (1) shortness of breath or dyspnea, with a respiratory rate of 30 breaths/min; (2) oxygen saturation (resting state) was 93% or less; and (3) $PaO^2/FiO^2 \leq 300$ mmHg.

The exclusion criteria were as follows: (1) age < 18 or > 70 years; (2) other organ transplant patients (liver, heart, lung, pancreas, bone marrow, etc.); (3) multiorgan transplant recipients; (4) time ≤ 2 months post-KTx; (5) merging rejection, tumor, posttransplant lymphoproliferative disorders (PTLD) or other infections; and (6) rituximab treatment.

After admission, the patient's first initial test was recorded before treatment. Peripheral blood samples were analyzed via flow cytometry within 24 h after collection. Symptomatic treatment of COVID-19 pneumonia patients after hospitalization included oxygen inhalation, nebulized expectoration, improvements in microcirculation, antipyretic action and anti-inflammatory effects, etc. Additionally, intravenous methylprednisolone or dexamethasone was administered to a limited number of patients. Some patients were treated with anti-COVID-19 drugs, including Azvudine, Nirmatrelvir/Ritonavir, and Monoravi. Among these patients, those receiving Nirmatrelvir/Ritonavir reduced or discontinued Tacrolimus because of the drug concentration.

Peripheral lymphocyte subsets panel

The assessment of PLSs was carried out using BD 6-color TBNK reagent and BD Trucount tubes to determine

the distribution and absolute numbers of lymphocytes from peripheral blood. These lymphocytes included CD3 + CD4 + T cells, CD3 + CD8 + T cells, NK cells, and CD19 + B cells. Every tube was stopped for testing when the count reached 10,000 cells. The collected flow results were collected and investigated via BD FACSCanto clinical software (BD Biosciences, San Jose, CA, USA).

Data and statistical analysis

Continuous data were expressed as the mean ± standard deviation (SD) and were compared using the unpaired *t* test. More than two groups would use one-way ANOVA. Variables that did not follow a normal distribution were presented as the median ± interquartile range (IQR) and were compared via the Mann–Whitney U test. Pearson's chi-square ($\chi 2$) test or Fisher's exact test was used to compare categorical data, where appropriate. Spearman correlation analysis was performed to identify relationships between the PLS and the clinical information of the patients [with $R > 0.4$ (moderate correlation) and $P < 0.05$]. The optimal cutoff values of indicators with obvious differences were calculated via receiver operating characteristic (ROC) curve analysis. The optimal cutoff values were calculated as "sensitivity + specificity − 1", and the maximum values were considered the optimal cutoff values. The risk factors for infection and disease progression after infection were screened via univariate and multivariate logistic regression analyses. Statistical calculations were performed via GraphPad Prism version 9.5 (GraphPad Software, Inc., La Jolla, CA, USA) or SPSS version 26.0 (SPSS, Inc., Chicago, IL, USA). The result was considered statistically significant when $P < 0.05$.

Results

Clinical characteristics

A total of 103 KTRs were included in this study, with 80 confirmed cases of COVID-19 pneumonia, which were divided into 55 MP patients and 25 SP patients. The remaining 23 KTRs were deemed stable patients for control purposes. Age, sex, CNI regimen and time since renal transplantation were not significantly different between the COVID-19 group and the stable group, indicating that the basic information of the two groups was consistent. The levels of platelets (PLTs), red blood cells (RBCs), lymphocytes (LYMs), Ca + and albumin (ALB) were lower in the COVID-19 group. Furthermore, the results revealed that the COVID-19 group tended to have weaker allograft renal function. On the other hand, the clinical characteristics of the MP and SP groups were not significantly different in uric acid (UA), white blood cell (WBC), and neutrophil (NEUT) counts. Moreover, the hospital stay (HS) of the MP group was generally shorter than that of the SP group. Patients in the MP group had better allograft renal function than those in the SP group did. A comparison of the results of routine blood tests revealed that the MP group had higher monocyte (MO), RBCs, LYMs, and PLTs counts than the SP group did, and there was no significant difference in WBCs among the non-transplanted, MP and SP groups. Meanwhile, lymphocytes showed kidney-transplanted patients had fewer LYMs, and even had fewer in severe pneumonia group. The levels of Ca + and ALB were greater in the MP group than in the SP group. Table 1 presents the details of these clinical characteristics.

Differences in the distributions and counts of PLSs

COVID-19 patients appear to have immunosuppression, which manifests as lymphopenia. The immune monitoring of PLSs revealed significant differences in the majority of the data. The absolute numbers (Ab No.) of CD3+, CD8+, and CD4 + T cells, B cells, and NK cells, as well as the percentages of CD4 + T cells, were significantly different in the regular immune monitoring panel between the COVID-19 group and the stable group. To further explore the correlation between PLS and COVID-19 in KTRs, a comparison between the mild subgroup and severe subgroup presented a similar case. In addition, compared with that in the MP group, the percentage of CD3 + T cells was obviously lower in the SP group. A comprehensive breakdown of the PLS indices can be found in Table 2; Fig. 1.

Correlation analysis of PLS and clinical information

We selected some indicators from the above results with statistical significance, and those with a correlation coefficient (CC) of less than 0.4 with other indicators were excluded. For the correlation analysis between different lymphocyte subpopulations and clinical information, we used Spearman correlation analysis since most variables did not follow a normal distribution. The correlation chart is shown in Fig. 2.

The immune function markers, which included the Ab No. of CD3 + T cells ($P < 0.001$, CC = 0.401) and CD4 + T cells ($P < 0.001$, CC = 0.417), were moderately positively associated with the eGFR. However, the red blood cell count ($P < 0.001$, CC = 0.506) was strongly correlated with the eGFR. In addition, two clinical indices had a moderate correlation with CD19 + B cells: the PLT ($P < 0.001$, CC = 0.535) and the level of Ca+ ($P < 0.001$, CC = 0.487). The analysis results also revealed that the Ab No. of CD8 + T cells was significantly correlated with the level of ALB ($P < 0.001$, CC = 0.498).

In COVID-19 patients, there was a positive correlation between the Ab No. of CD8 + T cells and the Ab No. of NK cells ($P < 0.001$, CC = 0.61). Additionally, the Ab No. of B cells was significantly positively correlated with

Table 1 Clinical characteristics between COVID-19-infected and stable allograft recipients

Characteristics	All($n=103$)	Stable($n=23$)	COVID-19($n=80$)			*P*-value[#]
			Mild($n=55$)	Severe($n=25$)	*P*-value[*]	
Age(years), mean ± SD	45.82 ± 9.56	44.00 ± 9.34	44.84 ± 9.30	49.64 ± 9.65	0.037[†]	0.304[†]
Male, n(%)	52(65.0%)	15(65.2%)	36(65.4%)	16(64.0%)	0.899[§]	0.985[§]
BMI(kg/m^2),mean ± SD	22.67 ± 3.17	24.03 ± 3.55	22.08 ± 2.82	22.45 ± 3.08	0.599[†]	0.004[†]
Time since transplantation (months), median ± IQR	65.0 ± 77.0	40.0 ± 77.0	71 ± 79	58 ± 99	0.868[Ψ]	0.139[Ψ]
Hospital stay period (days), median ± IQR	-	-	10 ± 5	23 ± 19	< 0.0001[Ψ]	-
CNI, n(%)					0.873[¶]	1.000[¶]
FK506	73(91.2%)	21(91.3%)	50(90.9%)	23(92.0%)		
CSA	7(8.7%)	2(8.7%)	5(9.1%)	2(8.0%)		
WBC(10^9/L), median ± IQR	6.35 ± 4.44	7.02 ± 3.30	6.30 ± 4.49	6.09 ± 6.95	0.554[Ψ]	0.905[Ψ]
PLT(10^9/L), median ± IQR	182.0 ± 99.0	214.0 ± 83.0	186.0 ± 99.0	139.0 ± 71.0	0.013[Ψ]	0.01[Ψ]
MO(10^9/L), median ± IQR	0.48 ± 0.34	0.48 ± 0.25	0.55 ± 0.36	0.43 ± 0.40	0.037[Ψ]	0.587[Ψ]
RBC(10^9/L), mean ± SD	3.85 ± 0.83	4.42 ± 0.64	3.96 ± 0.78	2.94 ± 1.13	0.004[Ψ]	< 0.0001
LYM(10^9/L), median ± IQR	0.93 ± 0.96	1.63 ± 1.03	0.87 ± 0.72	0.36 ± 0.71	< 0.0001[Ψ]	< 0.0001[Ψ]
NEUT(10^9/L), median ± IQR	4.76 ± 3.63	4.31 ± 1.95	4.50 ± 4.35	5.50 ± 6.39	0.176[Ψ]	0.157[Ψ]
Cr(μmol/L), median ± IQR	123.0 ± 118.0	86.0 ± 42.0	115.0 ± 110.0	204.0 ± 169.0	0.002[Ψ]	< 0.0001[Ψ]
BUN(mmol/L), median ± IQR	10.05 ± 9.33	7.10 ± 3.69	9.94 ± 10.50	15.38 ± 19.28	0.002[Ψ]	< 0.0001[Ψ]
UA(μmol/L), median ± IQR	323.0 ± 128.0	331.0 ± 75.0	313.0 ± 131.0	317.0 ± 160.0	0.537[Ψ]	0.105[Ψ]
eGFR(mL/(min*1.73m^2)),median ± IQR	59.94 ± 56.59	81.24 ± 24.47	63.39 ± 58.37	30.68 ± 31.39	0.002[Ψ]	0.001[Ψ]
Ca+(mmol/L)	2.28 ± 0.26	2.42 ± 0.16	2.27 ± 0.17	2.15 ± 0.22	0.015[†]	< 0.0001[Ψ]
Albumin(g/L)	35.07 ± 5.53	41.20 ± 3.82	34.1 ± 6.4	29.7 ± 5.4	0.001[Ψ]	< 0.0001[†]

SD standard deviation, *IQR* interquartile range, *CNI* calcineurin inhibitor, *CsA* cyclosporine A, *BUN* blood urea nitrogen, *Cr* serum creatinine, *UA* uric acid, *CRP* C reactive protein

[*] Comparison between the mild COVID-19 patients and the severe COVID-19 patients

[#] Comparison between the COVID-19 patients and the stable patients

[§] Tested by Chi-square test

[¶] Tested by Fisher's exact test

[Ψ] Tested by Mann-Whitney U test

[†] Tested by Welch's t-tested

Table 2 Regular immune monitoring panel of the patients

Parameters	All($n=103$)	Stable($n=23$)	COVID-19 patients			*P*-value[#]
			Mild($n=55$)	Severe($n=25$)	*P*-value[*]	
CD3 + T cells/TBNK, median ± IQR(%)	77.43 ± 14.65	78.63 ± 7.76	77.25 ± 12.23	73.29 ± 36.24	0.036[Ψ]	0.13[Ψ]
CD4 + T cells/TBNK, mean ± SD(%)	35.89 ± 11.78	36.60 ± 8.59	36.45 ± 11.26	29.43 ± 12.70	0.015[†]	0.002[†]
CD8 + T cells/TBNK, mean ± SD(%)	33.69 ± 12.31	33.69 ± 8.85	34.70 ± 10.10	31.26 ± 13.58	0.210[†]	0.806[Ψ]
B cells/TBNK, median ± IQR(%)	9.57 ± 9.59	9.53 ± 5.39	8.18 ± 12.87	12.77 ± 11.55	0.068[Ψ]	0.68[Ψ]
NK cells/TBNK, median ± IQR(%)	9.98 ± 9.01	9.98 ± 8.74	10.45 ± 9.78	7.74 ± 25.88	0.963[Ψ]	0.834[Ψ]
CD3 + T cells, median ± IQR(/μl)	560.0 ± 857.0	1283.0 ± 937.0	534 ± 652	179 ± 319	< 0.0001[Ψ]	< 0.0001[Ψ]
CD4 + T cells, median ± IQR(/μl)	269.0 ± 461.0	630.0 ± 488.0	324 ± 430	73 ± 142	< 0.0001[Ψ]	< 0.0001[Ψ]
CD8 + T cells, median ± IQR(/μl)	286.0 ± 361.0	562.0 ± 325.0	280 ± 262	73 ± 181	< 0.0001[Ψ]	< 0.0001[Ψ]
B cells, median ± IQR(/μl)	78.0 ± 114.0	175.0 ± 168.0	79 ± 112	47 ± 49	0.017[Ψ]	< 0.0001[Ψ]
NK cells, median ± IQR(/μl)	80.0 ± 154.0	155.0 ± 96.0	80 ± 149	37 ± 102	0.006[Ψ]	0.002[Ψ]
CD4/CD8 ratio, median ± IQR	1.07 ± 0.80	1.07 ± 0.80	0.45 ± 0.83	0.97 ± 0.76	0.383[Ψ]	0.052[Ψ]

[*] Comparison between the mild COVID-19 patients and the severe COVID-19 patients

[#] Comparison between the COVID-19 patients and the stable patients

[Ψ] Tested by Mann-Whitney U test

[†] Tested by Welch's t-tested

Cells/TBNK (%) – percentage of relevant cells in total T, B, and NK cells

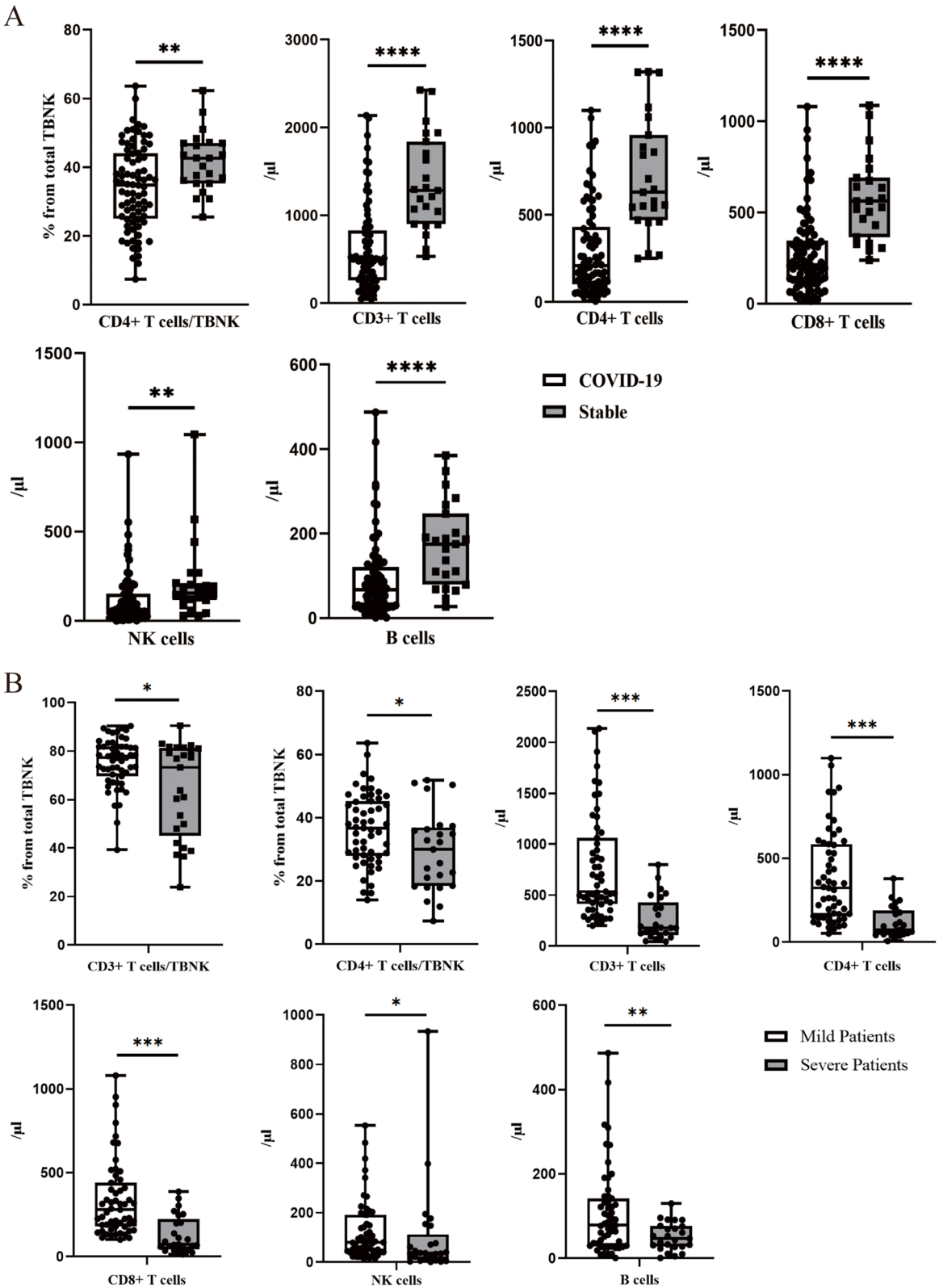

Fig. 1 **A** Distribution, percentage and absolute numbers (Ab No.) of TBNKs, as well as the CD4+/CD8 + T cell ratio, between COVID-19 patients and stable recipients. **** indicates $P < 0.0001$, *** indicates $P < 0.001$, ** indicates $P < 0.01$, and * indicates $P < 0.05$. **B** The distribution, percentage and Ab No. of TBNKs, as well as the CD4+/CD8 + T cell ratio, between mild and severe COVID-19 recipients. **** indicates $P < 0.0001$, *** indicates $P < 0.001$, ** indicates $P < 0.01$, and * indicates $P < 0.05$

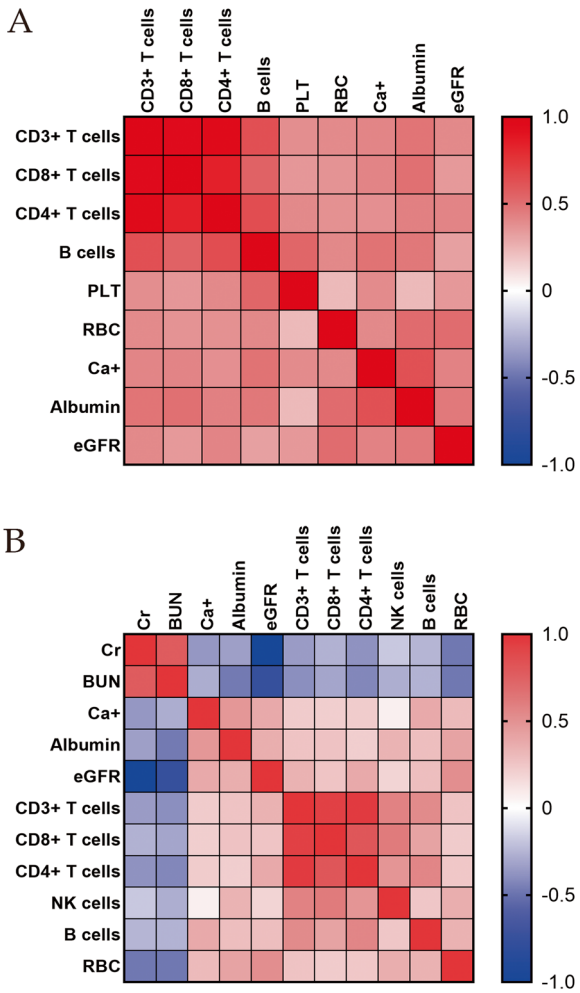

Fig. 2 **A** Correlation analysis of PLS with clinical information in all recipients. **B** Correlation analysis of PLS with clinical information in COVID-19 patients

the Ab No. of CD3+T cells ($P<0.001$, CC=0.53) and CD4+T cells ($P<0.001$, CC=0.56). We aimed to investigate the relationships between renal function indices and PLSs. However, the results revealed that renal function indices (serum creatinine and urea nitrogen) were weakly correlated with the Ab No. of CD4+T, CD8+T, and B cells ($P<0.05$, CC<0.4). We observed an inverse correlation between the BUN level and the Ab No. of CD4+T cells ($P<0.001$, CC=0.43) as well as the RBC count ($P<0.001$, CC=0.48). In addition, the ALB concentration was positively correlated with the CD4+T and B cell counts ($P<0.05$). The eGFR was positively correlated with the RBC count ($P<0.001$, CC=0.51).

ROC and cut-off values of PLS

We further analyzed the above PLS indicators with statistically significant differences to evaluate whether they have diagnostic value and determine the boundary point by ROC analysis. The data revealed that the Ab No. of the PLS had better diagnostic accuracy, especially the Ab No. of CD3+/CD8+/CD4+T cells. The results between the COVID-19 group and the stable group revealed that the area under the curve (AUC) were 0.865, 0.852, 0.854, 0.764 and 0.717 for Ab No. of CD3+T/CD8+T/CD4+T/CD19+B/CD16+CD56+NK cells, while the optimal cutoff values were 876.5, 288.5, 446, 102.5 and 111 (/μl), respectively.

On the other hand, by analyzing the differences between the MP and SP, we observed that the AUC of the Ab No. of CD3+/CD4+/CD8+T cells was greater than 0.7, while the Ab No. of CD3+T cells had the highest AUC (0.856), and the optimal cutoff value was 219.5/μl. The optimal cutoff values were 63.84, 24, 219.5, 113, 119.5, 40.5, and 99 for CD3+T cells/TBNK (%), CD4+T cells/TBNK (%), CD3+T cells(/μl), CD8+T cells(/μl), CD4+T cells(/μl), NK cells(/μl) and B cells(/μl), respectively. All the ROC curves are displayed in Fig. 3. In addition, Tables 3 and 4 shows the AUC and cut-off values.

Independent risk factors

Our study aimed to predict the independent risk factors for infection and disease progression on the basis of clinical descriptions and LPS. Above all, we investigated most of the clinical indices that were significantly related to disease progression via univariate logistic analysis, and the results are shown in Tables 5, 6, 7 and 8. However, only the level of albumin (OR=0.768, $P=0.007$)

Table 3 AUC and cut-off values of PLS in COVID-19 and stable recipients

Test result variable(s)	Cut-off value	SEM	Asymptotic 95% confidence		Sensitivity(%)	Specificity(%)	Youden index	AUC
			Lower bound	Upper bound				
CD4+T cells/TBNK(%)	30.565	0.055	0.573	0.786	0.413	0.957	0.37	0.679
CD3+T cells(/μl)	876.5	0.035	0.796	0.934	0.788	0.87	0.658	0.865
CD8+T cells(/μl)	288.5	0.038	0.778	0.926	0.638	0.9657	0.595	0.852
CD4+T cells(/μl)	446	0.037	0.781	0.928	0.763	0.87	0.633	0.854
NK cells(/μl)	111	0.057	0.606	0.829	0.675	0.783	0.458	0.717
B cells(/μl)	102.5	0.053	0.660	0.869	0.713	0.739	0.452	0.764

SEM – standard error of mean. /μl – absolute number of cells

Table 4 AUC and cut-off values of PLS in MP and SP

Test result variable(s)	Cut-off value	SEM	Asymptotic 95% confidence		Sensitivity(%)	Specificity(%)	Youden index	AUC
			Lower bound	Upper bound				
CD3 + T cells/TBNK(%)	63.84	0.073	0.505	0.789	0.48	0.891	0.371	0.647
CD4 + T cells/TBNK(%)	24	0.07	0.524	0.798	0.44	0.891	0.331	0.661
CD3 + T cells(/μl)	219.5	0.047	0.764	0.948	0.64	0.982	0.622	0.856
CD8 + T cells(/μl)	113	0.052	0.731	0.936	0.64	0.964	0.604	0.833
CD4 + T cells(/μl)	119.5	0.045	0.763	0.939	0.68	0.873	0.553	0.851
NK cells(/μl)	40.5	0.07	0.554	0.829	0.6	0.8	0.4	0.691
B cells(/μl)	99	0.06	0.55	0.784	0.96	0.418	0.378	0.667

SEM – standard error of mean. /μl – absolute number of cells

was strongly related to COVID-19 infection according to the univariate logistic analysis. There was a strong multiple collinearity problem between the Ab No. of CD3+, CD8+, and CD4 + T cells according to the collinearity judgment in the logistic regression of risk factors for PLS. Therefore, these variables were not included in the multivariate logistic analysis. The results revealed that the percentage of CD4 + T cells (OR = 0.918, $P = 0.003$), the Ab No. of NK cells (OR = 0.996, $P = 0.026$) and B cells (OR = 0.994, $P = 0.016$) were independent risk factors for infection with COVID-19 in the multivariate logistic model of PLS.

In the present study, only hospital stay duration (OR = 1.184, $P = 0.001$) among the clinical indices was an independent risk factor for disease progression in the multivariate logistic analysis of the COVID-19 group. According to the results of the univariate logistic analysis, the percentages of CD3 + and CD4 + T cells, as well as the Ab No. of CD3+, CD8+, CD4 + T and B cells, were significantly associated with disease progression. The PLS multivariate logistic model included the percentage of CD3 + and CD4 + T cells, as well as the Ab No. of B cells. Finally, the percentages of CD3+ (OR = 0.916, $P = 0.004$), CD4+ (OR = 0.929, $P = 0.009$) T cells and the Ab No. of B (OR = 0.980, $P = 0.007$) cells were independent risk factors for COVID-19 progression.

Discussion

This retrospective study effectively outlined the distribution and Ab No. of PLSs and illustrated their relevance in severe COVID-19-infected patients after KTx. Our findings revealed a substantial decrease in the percentages of CD3 + and CD4 + T cells, along with the Ab No. of TBNK cells after COVID-19 infection and worsening illness conditions in KTRs. Additionally, the percentages of CD3 + and CD4 + T cells, as well as the Ab No. of B cells, were independent risk factors for severe pneumonia progression after KTx. These results support the notion that COVID-19 infection and aggravation could cause damage to T lymphocytes and their subsets. Therefore, monitoring PLS may provide a comprehensive understanding of the basic immune status for individualized prevention and treatment in COVID-19-infected patients after KTx. Overall, monitoring PLSs is important for long-term KTRs.

The clinical data from numerous studies have consistently demonstrated obvious distinctions between individuals experiencing mild and severe cases of COVID-19, with age and HS exhibiting more pronounced disparities among them [10]. The results of the age differences suggested that elderly people represent a large at-risk population. The cause might be associated with age-dependent variations in the initiation of the adaptive immune response against SARS-CoV-2 [11].

Lymphocytes and their subsets are integral in preserving immune balance and orchestrating the inflammatory response within the host, including the involvement of NK cells, monocytes, and neutrophils in innate immune responses, along with CD4+/CD8 + T cells and B cells in adaptive immune responses. The antiviral process relies significantly on adaptive immunity, particularly T cells, for crucial functions [12]. Like other severe infections caused by viruses, cellular immune disorders and inflammatory cytokine storms rapidly progress after COVID-19 infection [13]. During immune resistance against COVID-19, CD4 + T cells are presumed to contribute to antibody generation, with follicular helper T cells correlated with humoral immunity during the memory phase [14]. A study conducted by Tan AT revealed the crucial role of CD8 + T cells in mitigating the severity of COVID-19, facilitating rapid viral clearance, and inducing long-term immune protection [15]. During the early stage of the COVID-19 pandemic, numerous studies on the T-cell profile in non-transplant populations with COVID-19 have shed light on the occurrence of lymphocytopenia [16, 17]. Changes in lymphocyte subsets are

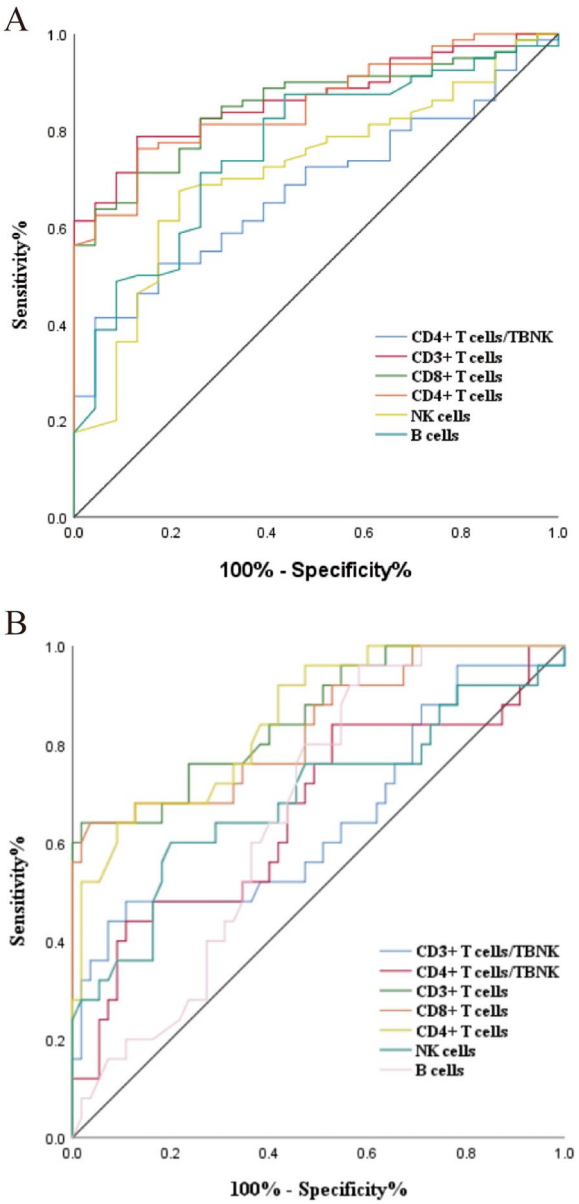

Fig. 3 **A** ROC curve of PLS in COVID-19 patients and stable recipients. **B** ROC curve of PLS for MP and SP

linked to the severity of COVID-19, with critically ill and deceased patients exhibiting extremely low CD4+and CD8+T cells [18]. In addition, multiple lines of evidence support the correlation between the severity of COVID-19 and not only a decrease in the circulating lymphocyte count but also a decrease in T cell activity, particularly in the production of cytokines such as IL-2 and IFN-γ [19].

Immunosuppression seems to have a more negative impact on immune function after contracting COVID-19, especially in solid organ transplant (SOT) recipients [20, 21]. KTRs face an increased risk of immune impairment following COVID-19 infection, which is attributed to factors such as immunosuppression, underlying chronic kidney disease, and additional comorbidities, notably diabetes and hypertension [22]. Our study revealed that patients with severe COVID-19 following KTx tended to exhibit more pronounced immune injury. On the other hand, there is a possibility that immunopathogenesis could be a significant factor in the development of severe COVID-19. Although a few international single centers have conducted related research examining immune function in KTRs following COVID-19 infection [23, 24], data from China are incomplete. Therefore, the findings of this study might be valuable for Chinese organ transplant doctors. Exploring the pathogenesis of the immune system in patients with COVID-19, particularly immunosuppressed patients, is challenging. The mechanism by which cell-mediated immunity contributes to worsen the severity of COVID-19 are not fully understood and need

Table 5 A risk factors of clinical information for infection by logistic regression in all recipients

Variable	Univariate analysis OR (95% CI)	*P*	Multivariate analysis OR (95% CI)	*P*
Age(years)	1.027(0.977, 1.079)	0.301		
BMI	0.796(0.677,0.936)	0.006		
PLT(10^9/L)	0.993(0.987,0.999)	0.031		
MO	0.912(0.191,4.351)	0.908		
RBC	0.279(0.135,0.577)	0.001		
LYM	0.120(0.047,0.305)	< 0.001		
eGFR	0.972(0.955,0.989)	0.001		
Ca+	0.001(0.000,0.043)	< 0.001		
Alb	0.692(0.595,0.804	< 0.001	0.768(0.634,0.930)	0.007

Table 6 B risk factors of clinical information for progression by logistic regression in COVID-19 patients

Variable	Univariate analysis OR (95% CI)	*P*	Multivariate analysis OR (95% CI)	*P*
Age(years)	1.0057 (1.002, 1.116)	0.042		
Hospital stay period(days)	1.176(1.083,1.278)	< 0.001	1.184(1.075,1.305)	0.001
PLT(10^9/L)	0.988(0.979,0.997)	0.009		
MO(10^9/L)	0.161(0.026,0.994)	0.049		
RBC(10^9/L)	0.42(0.211,0.833)	0.013		
LYM	0.113(0.029,0.441)	0.002		
Cr	1.005(1.001,1.009)	0.028		
BUN	1.067(1.019,1.118)	0.006		
Ca+	0.041(0.003,0.598)	0.02		
Alb	0.829(0.726,0.946)	0.006		

Table 7 A risk factors of PLS for infection by logistic regression in all recipients

Variable	Univariate analysis OR (95% CI)	*P*	Multivariate analysis OR (95% CI)	*P*	VIF
CD4 + T cells/TBNK(%)	0.943(0.902,0.987)	0.011	0.918(0.867, 0.971)	0.003	3.076
CD3 + T cells(/μl)	0.998(0.997,0.999)	< 0.001			151.780
CD8 + T cells(/μl)	0.995(0.993,0.997)	< 0.001			41.602
CD4 + T cells(/μl)	0.996(0.994,0.998)	< 0.001			54.564
NK cells(/μl)	0.997(0.994,1.000)	0.032	0.996(0.992,0.999)	0.026	1.742
B cells(/μl)	0.992(0.988,0.997)	0.002	0.994(0.989, 0.999)	0.016	1.921

Table 8 B risk factors of PLS for progression by logistic regression in COVID-19 patients

Variable	Univariate analysis OR (95% CI)	*P*	Multivariate analysis OR (95% CI)	*P*	VIF
CD3 + T cells/TBNK(%)	0.948(0.915,0.983)	0.003	0.916(0.863, 0.972)	0.004	4.232
CD4 + T cells/TBNK(%)	0.949(0.909,0.992)	0.019	0.929(0.88, 0.981)	0.009	4.568
CD3 + T cells(/μl)	0.994(0.991,0.997)	< 0.001			192.016
CD8 + T cells(/μl)	0.989(0.983,0.995)	< 0.001			54.733
CD4 + T cells(/μl)	0.989(0.984,0.995)	0.001			70.928
NK cells(/μl)	0.999(0.995,1)	0.534			2.635
B cells(/μl)	0.987(0.976,0.997)	0.014	0.980(0.966, 0.995)	0.007	2.346

further research and exploration. Thus, whether immune cell responses from KTxs can enhance protection against COVID-19 infection is highly important for predicting the severity of disease progression.

The kidney is vulnerable to COVID-19, which was confirmed by the risk map of other organs susceptible to SARS-CoV-2 invasion on the basis of ACE2 expression via single-cell RNA sequencing datasets [25]. Given the influence of COVID-19, it is imperative to focus on the renal graft function of KTRs in the field of transplant surgery. The kidney allograft faces an increased risk of acute kidney injury (AKI) due to transplant-related factors such as graft rejection and CNI toxicity. Moreover, AKI frequently occurs after COVID-19 infection and has the potential to induce or exacerbate chronic kidney diseases, including glomerular diseases. The progression of COVID-19 exacerbates, ultimately resulting in diminished graft function in KTRs [26]. Notably, renal function was affected more in KTRs with COVID-19, and this finding was related to severity. The key factors contributing to kidney injury in patients with COVID-19 include direct injury because of COVID-19, hemodynamic insults, cytokine-related injury, coagulation dysfunction and immune dysregulation [27]. Disruption of the renin–angiotensin system (RAS) may be a key factor affecting renal graft function. Research has indicated that COVID-19 infection coincides with a reduction in ACE2 expression, resulting in an increase in Ang II levels and possible overactivation of the RAS. The RAS imbalance in COVID-19 patients may increase arterial pressure and acute tubular necrosis, further causing damage to grafted renal tissue. There is evidence indicating that the graft function of KTRs may not completely recover following AKI caused by COVID-19. Furthermore, the existence of proteinuria at baseline has autonomous prognostic value in predicting incomplete graft recovery [28]. Dysregulated immune responses in COVID-19 are an important mechanism leading to impaired renal function [29], which is one of the aims of our study. Unrestrained inflammation may hinder viral clearance by inducing T-cell exhaustion in particular cases [30]. However, the relationship between immune function and graft function remains unclear. Unfortunately, there was no evidence of a correlation between lymphocyte count and graft function in our analysis. Therefore, larger and longer-term cohort studies investigating the effects of KTx on immune function and renal function in patients with COVID-19 are urgently needed.

This research has several limitations. First, this was a retrospective, small, single-center, cross-sectional clinical study. This might limit the applicability of the study's findings. This was a small sample study including 80 KTRs following COVID-19 infection, which might have confounded the results and potentially

introduced selection bias. Second, mild COVID-19 patients were mainly young, whereas patients with severe COVID-19 were mainly elderly. In addition, it is difficult to determine the actual onset time, which leads to differences in the collection times of the PLS data. Furthermore, we lacked information on patients receiving the COVID-19 vaccine and the symptoms at diagnosis. Finally, our study was limited by the lymphocyte count and was not focused on the specific immune functions of lymphocytes, such as the expression of cytokines and T cell activation.

Despite the above, our research still plays a positive role. In our previous study, the association between immune monitoring and pneumonia in KTx patients was demonstrated in detail by applying machine learning models, and results revealed that PLS is important for the diagnosis and prognosis of pneumonia patients after kidney transplantation. Similarly, immune function monitoring has greatly contributed to better individualized therapy for COVID-19-infected KTRs.

Conclusion

This study successfully detailed the distribution and Ab No. of PLSs and demonstrated their significance in severe COVID-19-infected patients after KTx. The percentage of CD3 + T cells had good diagnostic efficacy for disease progression after COVID-19 pneumonia. Additionally, percentages of CD3 + and CD4 + T cells, as well as the Ab No. of B cells, were independent risk factors for severe pneumonia progression after KTx. On the basis of monitoring peripheral lymphocyte subpopulations, better individualized prevention and therapy might be achieved for patients with severe COVID-19 pneumonia after KTx.

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Disclosure statement
No potential conflicts of interest were reported by the author(s).

Authors' contributions
Yingzi Ming, Ke Cheng, Quan Zhuang and Jiang Zhu designed the research. Quan Zhuang, Bo Peng and Jiang Zhu performed the experiments. Quan Zhuang and Jiang Zhu collected and analyzed the data. Bo Peng and Yi Zhu provided guidance on the experiment and technical support. Yingzi Ming, Quan Zhuang and Jiang Zhu drafted the manuscript. All the authors contributed to the article and approved the submitted version.

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Data availability
All the data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
Written informed consent was obtained from each participant to participate in the study. This study adhered to the Helsinki Declaration and received approval from the Institutional Review Board of Third Xiangya Hospital, Central South University (IRB No.21176).

Competing interests
The authors declare no competing interests.

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