Post-transplant monitoring of lymphocyte counts predict the occurrence of CMV DNAemia in early phase of kidney transplantation

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Abstract

Background The aim of this study was to assess whether monitoring of the number of lymphocytes in peripheral blood was helpful for evaluating the risk of cytomegalovirus (CMV) infection after kidney transplantation.

Methods The total numbers of lymphocyte in peripheral blood were measured at baseline and posttransplant months 1, 3 and 6. Risk factors for DNAemia in KTRs were analyzed using univariate logistic regression analyses. Areas under receiver operating characteristic (ROC) curves were applied to assess the accuracy of lymphocyte counts for predicting CMV DNAemia.

Results After follow-up 6 months, CMV replication was detected in 12 (31.6%) kidney transplant recipients (KTRs). The total lymphocyte counts were significantly decreased in KTRs with CMV DNAemia in 1, 3 and 6 months. There was a negative correlation between CMV copies and the lymphocyte counts in 1, 3 and 6 months post-transplantation, and the decrease of lymphocyte counts in the 6 months post-transplantation was the risk factor of CMV DNAemia in the KTR. Patients with lymphocyte counts 1.085×10^9 cells/L had higher cumulative incidence of CMV DNAemia.

Conclusions The lymphocyte counts post kidney transplantation may be used as a simple and effective indicator for monitoring the CMV DNAemia status in KTR and for predicting the risk of CMV DNAemia.

Background

Cytomegalovirus (CMV) belongs to the β -herpesvirus family and can infect different species including rodents, non-human primates, and humans. Although CMV is widely distributed in the general population with a high prevalence, the primary CMV infection is usually asymptomatic and harmless [1]. In contrast, reactivation of latent CMV infection may cause CMV disease and be a major cause of morbidity and mortality in immunocompromised kidney transplant recipients (KTR). Cumulative data confirmed that CMV infection could result in the increase of the incidence of acute rejection and chronic histological changes of kidney such as interstitial fibrosis and tubular atrophy [2, 3]. If without any form of preventive strategies, CMV infection primarily occurs in the first 3 months following transplantation, and the incidence of symptomatic CMV infection reached to 20-60%. Moreover, the severity of CMV infection depends on the serostatus of the recipient (R) and donor (D) before transplantation, transplanted organ type, and immunosuppressive treatment after kidney transplantation [4].

The reactivation of CMV in KTR that commonly occurs shortly after transplantation is the consequence of a temporary disruption of an existing balance between immunological surveillance and viral replication by treated with immunosuppressive agents and by systemic infection and inflammation [5]. Clinical manifestations of CMV disease in KTR are non-specific and commonly include fever with flu-like syndrome, gastrointestinal disorders and hematologic and respiratory manifestations. Currently, use of specific antiviral drugs such as valaciclovir and ganciclovir significantly improve the prognosis of CMV

infection and graft survival. Both universal prophylaxis and preemptive therapy have alleviated the impact of CMV on graft function.

In recent years, some immune-monitoring strategies have been applied to evaluate the risk of CMV infection in KTR, the dynamics of certain cells in peripheral blood have been confirmed to be valuable for predicting opportunistic infection in recipients who received kidney and heart transplantation [6, 7]. Moreover, some CMV-specific T-Cell immunity was reported to associate with the CMV infection [8-10]. However, one more simple surrogate approach would be needed to monitor the state of immunosuppression, and further estimate the possibility of CMV infection in KTR. Therefore, we try to analyze the effect of CMV DNAemia within the first 6 months on graft survival and function in KTR, and explore the latent value of routine monitoring of total lymphocyte counts in peripheral blood to predict the occurrence of CMV infection in the present study.

Methods

Patients

A total of 38 KTR with recipient (R) and/or donor (D) positive CMV serology were enrolled in this study and signed written informed consent from November 2015 to December 2016. This study was approved by the ethics committee of Beijing Chao-Yang Hospital, Capital Medical University and was conducted in accordance with the Declaration of Helsinki (World Medical Association). All KTRs included in this study underwent kidney transplantation in the institute of urology and nephrology, Beijing Chao-Yang hospital, and had a functional allograft were eligible to participate the follow-up in the out-patient clinic. The standard immunosuppressive regimens consisted of basiliximab or anti-thymocyte globulins induction followed by maintenance therapy with mycophenolate (500-1000 mg/day), prednisone and tacrolimus(3-8 mg/day).

CMV prevention and monitoring

38 patients were treated with ganciclovir (500mg, oral administration, tid) or valganciclovir (450mg, oral administration, qd) universal prophylaxis for 3 months. PCR for CMV DNAemia was performed at 2-week intervals for the first 3 months, and 1-month intervals at 4, 5 and 6 months thereafter. CMV DNAemia was defined by detection of a viral load of >500 copies/ml. All patients were followed up prospectively for 6 months or until death or graft failure.

Statistical analysis

SPSS version 21.0 (SPSS Inc., Chicago, IL, USA) was applied to perform all statistical analyses. Quantitative variables are expressed as mean and standard deviation and qualitative variables were described by frequency (percentage). Quantitative variables were compared between the two CMV infected and non-infected groups by using independent samples t-test and qualitative variables were analyzed by using Chi-square test. Risk factors for CMV DNAemia in KTR were analyzed using univariate

logistic regression analyses. Receiver operating curve (ROC) analysis was performed to calculate the specificity and sensitivity of lymphocyte levels for detection of CMV infection. A *P* value <0.05 was considered significant.

Results

Study population and incidence of CMV DNAemia

Data of 38 KTR receiving universal prophylaxis for anti-CMV treatment were included in this study. CMV replication was detected in 12 (31.6%) transplanted recipients within the first 6 months, 6 patients were infected with CMV in the first month, 6 patients were infected with CMV in the third month, and CMV DNAemia happened in 8 patients after 6 month kidney transplantation, respectively.

Demographic and baseline clinical characteristics of patients pre-transplantation are presented in Table 1. There was no comparable regard to the baseline characteristics including recipient age, gender, weight, underlying disease, induction therapy, last control immune suppression and incidence of delayed graft function (DGF) in patients with CMV DNAemia or not. Similarly, there was no significant difference in hemoglobin, white blood cells, neutrophil, lymphocyte, serum creatinine, blood urea nitrogen (BUN), albumin and uric acid between two groups (Table 2).

Dynamics of lymphocyte during the 6 months after kidney transplantation

In order to determine the effect of CMV DNAemia on KTR, we further analyzed their biochemical parameters at 1, 3 and 6 months post-transplantation. As is shown in figure 1, we found that the lymphocyte counts were significantly higher in recipients free from CMV DNAemia in 1, 3 and 6 months when compared to those who developed CMV DNAemia. Meanwhile, the level of serum creatinine significantly increased gradually.

Lower lymphocyte counts were the risk factor of CMV DNAemia in the KTRs

Table 3 showed that there was a negative correlation between CMV copies and the lymphocyte counts in 1, 3 and 6 months post-transplantation among the 38 KTR, although this was not statistically significant in the third month. In the univariate analysis, the decrease of lymphocyte counts in the 6 months post-transplantation was the risk factor of CMV DNAemia in the KTR (Table 4).

Decrease of lymphocyte counts is a predictor of CMV DNAemia in the KTR

According to the analysis of ROC, the lymphocyte counts were confirmed to be the most accurate parameter at month 6 for predicting CMV DNAemia (AUC=0.78, 95% CI: 0.69-0.96, *P* = 0.005). On the basis of these statistical curves, we selected the cutoff value of 1.085×10^9 lymphocytes/L with the highest Youden's index. At the optimal cutoff value of lymphocyte counts, the sensitivity was 80.7% and specificity was 75.0% (figure 2).

Discussion

In our present study, we demonstrated that monitoring of the number of lymphocytes in peripheral blood during the early period post kidney transplantation should be useful for predicting the occurrence of CMV DNAemia in KTRs. Especially, patients with a lymphocyte counts $< 1.085 \times 10^9$ cells/L at month 6 experienced a higher incidence of CMV DNAemia compared with those above this cutoff, which can predict the occurrence of CMV DNAemia with sensitivity and specificity above 75%. In addition, we also found that there was a negative correlation between CMV copies and the lymphocyte counts in post kidney transplanted patients.

Monitoring of cell-mediated immunity (CMI) has been considered to be a promising strategy to reduce the incidence of opportunistic infection in KTRs. This monitoring could be based on quantitative measurement of the counts of total lymphocyte. A few studies have reported that low lymphocyte counts were associated with the adverse events in solid organ transplantation patients. Calarota SA et al. found that heart transplant recipients developing opportunistic infections had lower CD4⁺ and CD8⁺ T cells than those without infections, and the opportunistic infections only happened in the KTRs with low CD8⁺ T cells [6]. In liver transplantation recipients, the pretransplant total lymphocyte counts were significantly lower in patients with an infection compared with those without one, and there was a significant correlation between the risk of development of a posttransplant infection and pretransplant total lymphocyte counts $< 1.00 \times 10^3 / \mu\text{l}$ [11]. Results from Fernández-Ruiz M et al. also showed that CD8⁺ T cell count $< 0.100 \times 10^3 / \mu\text{l}$ at one month after transplantation can predict the subsequent occurrence of overall opportunistic infection and CMV disease in the non-ATG induction group, and CD4⁺ T cell count at month 1 $< 0.050 \times 10^3 / \mu\text{l}$ showed negative predictive potential for the subsequent occurrence of overall opportunistic infection and CMV disease in the ATG induction group respectively [7]. In addition, Ducloux D et al. found that lower CD4⁺ T cell level was associated with increased risk of noncutaneous neoplasia in KTRs [12], and they subsequently confirmed that CD4⁺ T cell lymphopenia persisting for > 1 year resulted in a higher death rate in KTRs, which associated with a nearly five-fold risk for death [13]. Various studies also reported the correlation of lymphocyte counts with pneumocystis pneumonia (PJP) infection. Lee SH et al. showed that the lymphocyte counts were relatively low in the PJP group, and the univariate analysis suggested the decrease of lymphocyte counts were associated with the development of PJP infection [14]. Thus, posttransplant kinetics of lymphocyte acted as a simple indicator for monitoring the immune condition in these solid organ transplanted patients.

Although the data from Nierenberg NE et al. suggested that pretransplant lymphopenia is a novel independent predictor of both CMV disease and non-CMV invasive infections after liver transplantation [15], Fernández-Ruiz M et al. also demonstrated that absolute lymphopenia evaluation pre-transplantation was an independent risk factor for post-transplant infection in liver transplanted recipients [11]. An earlier study showed the combination of CMV infection and a previous episode of acute rejection was related with increased vasculopathic changes in 6-month protocol biopsy specimens [16]. Meanwhile, the other study confirmed that CMV DNAemia with viral load of more than or equal to 2000

copies/mL could result in increase of the risk of interstitial fibrosis and tubular atrophy in protocol biopsy within the first 3 months after transplantation [17]. We did not find any differences in lymphocyte counts before kidney transplantation in our study, which indicated that there was no association between baseline lymphocyte counts and the risk of CMV DNAemia. Thus, we could not determine the predictive value of lymphocyte counts pre-transplantation.

There are some limitations in our study. We only evaluated the correlation of lymphocyte counts with the CMV DNAemia in early phase of kidney transplanted recipients, the long term affection lymphocyte counts of on CMV DNAemia did not be further elucidation. Moreover, we only detected the total lymphocyte counts, but we did not perform any functional studies of different lymphocytes subgroup. Finally, due to it was a retrospective observational study and single center cohort, the sample size was small, and making it unlikely to perform other subgroup analyses.

Conclusion

In conclusion, low lymphocyte counts early after renal transplantation was associated with CMV DNAemia, which may associate with significant loss of renal function. We suggest that post-transplant decrease of lymphocyte counts in peripheral blood could be as a rapid, simple and effective biomarker, providing a convenient alternative way to notice the risk of CMV DNAemia.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Beijing Chao-Yang Hospital, Capital Medical University (2017-047) and was conducted in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Authors' contribution was described as Study Design (A), Data Collection (B), Statistical Analysis (C), Data Interpretation (D), Literature Search (E), manuscript draft (F), Critical revision of the manuscript for scientific and factual content (G) and Funds Collection (H) in the study. Each author's contribution to the study were, SF for A, B, C, D, E and F, JY for A, C, E, and XZ for D, E, G and H respectively. XZ was the corresponding author.

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Tables

Table 1. Demographic characteristics of the kidney transplant recipients.

Demographic Data	CMV negative	CMV positive	P value
Age	42.7±12.5	43.7±14.1	0.8269
Gender			0.8389
Male	17	9	
Female	9	3	
Weight	66.1±13.3	67.3±15.6	0.8080
Underlying disease			0.4188
Chronic nephritis	23	9	
Hypertension	1	0	
IgA nephropathy	2	1	
Polycystic kidney disease	0	1	
Diabetes mellitus	0	1	
Induction therapy			0.5298
ATG	13	9	
Basiliximab	10	4	
No induction	3	0	
Last control immune suppression			0.9671
MP+cellcept+Tac	14	7	
MP+myfortic+Tac	12	5	
DGF			
Yes	1	0	0.7890
No	25	12	

Table 2. Comparison of biochemical indicator pre-kidney transplantation between the CMV infection and CMV non-infection patients.

Laboratory Data	CMV negative	CMV positive	<i>P</i> value
Hemoglobin, g/L	110.6±17.2	120.3±13.5	0.0940
White blood cells, × 10 ⁹ /L	7.2±1.8	6.6±1.4	0.3152
Neutrophil, × 10 ⁹ /L	4.8±1.8	4.7±1.2	0.8623
lymphocyte, × 10 ⁹ /L	1.5±0.5	1.4±0.4	0.5474
Serum creatinine, (mmol/L)	735.9±282.0	799.1±246.9	0.5094
BUN, mmol/L	19.5±8.2	20.1±6.6	0.8256
Albumin, g/L	39.4±5.6	39.3±6.4	0.9612
Uric acid, mmol/L	304.2±131.4	348.4±86.7	0.2964

Table 3. The negative correlation of CMV copies and lymphocyte counts after 1, 3 and 6 months post-transplantation.

Lymphocyte, × 10 ⁹ /L	CMV Copies		
	R	95% CI	<i>P</i> value
1 m	-0.8270	-0.9805 to -0.0468	0.0423
3 m	0.6891	-0.5318 to 0.8213	0.1981
6 m	-0.7559	-0.9365 to -0.1076	0.0300

Table 4. Decrease of lymphocyte counts after 6 months of kidney transplantation is the risk factor of CMV DNAemia.

Lymphocyte	Univariate analysis Odds ratio (95% confidence interval)	P value
1 m	0.151(0.020-1.109)	0.151
3 m	0.044(0.002-1.235)	0.066
6 m	0.048(0.003-0.675)	0.024

Figures

Figure 1

Changes of lymphocyte counts and serum creatinine in kidney transplant recipients with or without CMV DNAemia. A. The lymphocyte counts were significantly higher in recipients free from CMV DNAemia after 1, 3 and 6 months kidney transplantation when compared to those who developed CMV DNAemia (* $P < 0.05$ vs. CMV negative group); B. The level of serum creatinine significantly increased after 6 months kidney transplantation (* $P < 0.05$ vs. CMV negative group).

Figure 2

ROC curve analysis by lymphocyte counts to predict CMV DNAemia in kidney transplant recipients. A. ROC curve by using lymphocyte counts to predict CMV DNAemia after 1 month kidney transplantation (AUC=0.75, 95% CI: 0.54-0.96, P=0.05); B. ROC curves for lymphocyte counts to predict CMV Anaemia after 3 month kidney transplantation (AUC=0.75, 95% CI: 0.54-1.00, P=0.05); C. ROC analysis of the lymphocyte counts to predict CMV DNAemia at 6 month kidney transplantation (AUC=0.78, 95% CI: 0.69-0.96, P=0.005).