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Usefulness of Monitoring Cell-Mediated Immunity for Predicting Post–Kidney Transplantation Viral Infection

K. Nishikawa, S. Mizuno, S. Masui, H. Kanda, Y. Yamada, K. Arima, S. Isaji, and Y. Sugimura

ABSTRACT

Background. Monitoring cell-mediated immunity (CMI) can be used to estimate the risk of viral infections in kidney transplant recipients. The Immuknow (IMK) assay measures $CD4^+$ T-cell adenosine triphosphate activity, assesses patient CMI status, and assists clinicians in determining the risk of viral infection.

Methods. We retrospectively analyzed 224 IMK values in 39 kidney transplant recipients at our institution from April 2012 to January 2013. We analyzed the relationship between IMK value and viral infection during the early and late post-transplantation periods. Multiple regression analyses were performed, to determine which factors impacted the results of the IMK assay.

Results. Eight patients developed viral infections, including BK virus, cytomegalovirus, herpes simplex, and shingles. Five infections occurred in the early post-transplantation period (<50 d) and 3 in the late period (>120 d). The IMK levels in patients who developed an infection in the early period were within normal limits; however, those in the late period were significantly lower than 200 ng/mL (421.0 ± 062.6 for early vs 153.7 ± 72.7 for late; $P = .02$). Our multiple regression analyses indicated that peripheral white blood cell and neutrophil counts affected IMK values ($P = .03$ and $P = .02$, respectively).

Conclusions. The IMK assay is a useful test for identifying patients at risk for post-transplantation viral infections in the late transplant period.

THE DEVELOPMENT of new immunosuppressive agents has dramatically improved graft acceptance and patient survival in kidney transplant patients. Nevertheless, viral infections, including cytomegalovirus (CMV), BK virus (BKV), EB virus (EBV), and Herpes zoster, etc, are important complications that affect patient morbidity and mortality. T cells, especially $CD4^+$ T cells, are the primary population of immune cells that mediate allograft rejection and protect the host from infection from a variety of pathogens, including viruses [1]. In 2002, the US Food and Drug Administration approved the Cyleximmune cell function assay (Immuknow [IMK]) for assessing cell-mediated immunity (CMI) in immunosuppressed populations by measuring intracellular adenosine triphosphate (ATP) in stimulated $CD4^+$ T cells [2]. Some studies have shown that IMK is useful for predicting viral infections in renal transplant recipients [3–6], whereas other studies have not [7–9]. In the present report, we investigated the relationship between virus infection and IMK value, and factors influencing IMK values in kidney transplant patients.

METHODS

Samples and Patients

We retrospectively analyzed 224 IMK values in 39 kidney transplant recipients at our institution from April 2012 to January 2013.

From the Department of Nephro-Urologic Surgery and Andrology (K.N., S. Masui, H.K., Y.Y., K.A., Y.S.) and Department of Hepatobiliary Pancreatic and Transplant Surgery (S. Mizuno, S.I.), Mie University School of Medicine, Mie, Japan.

Address reprint requests to Kouhei Nishikawa, MD, Department of Nephro-Urologic Surgery and Andrology, Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu, Mie 514-8507, Japan. E-mail: kouheini@clin.medic.mie-u.ac.jp

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Although both CMV antigenemia and disease were defined as CMV infection, primary CMV infection was not included in this study. BKV infection was defined as BKV proliferation in urine or serum according to polymerase chain reaction.

Multiple regression analyses were performed to determine which factors affected IMK values. Age, sex (male vs female), postoperative days, use of tacrolimus and mycophenolate mofetil (yes vs no), white blood cell, neutrophil, lymphocyte and monocyte counts (/μL), and C-reactive protein (CRP; mg/dL) were included as variables. In addition, we analyzed the relationship between IMK value and post-transplantation period according to viral infection state.

IMK results were not used to manage patients. Clinical and follow-up data were obtained from medical records. Each patient gave full informed consent. The Institutional Review Board of Mie University School of Medicine approved the study protocol.

Immuknow Assay

Peripheral blood samples were collected into a sodium heparin tube and the intracellular ATP level measured. All blood samples were processed on the day of sample collection. Briefly, 250 mL of anticoagulated whole blood was diluted with the provided sample diluent to a final volume of 1,000 μL. Samples were added to 96-well plates and incubated for 15–18 hours with phytohemagglutinin in an incubator (37°C, 5% CO_2). After enrichment of $CD4^+$ T cells by the addition of magnetic particles coated with anti–human CD4 monoclonal antibody (Dynabeads; Dynal, Oslo, Norway), lymphocytes were washed and lysed to release intracellular ATP. Released ATP was measured with a luciferin/luciferase assay in a luminometer.

Statistical Analysis

$P < .05$ was considered to be statistically significant for multiple regression analyses. SPSS 18.0 software (SPSS, Chicago, Illinois, USA) was used for statistical analysis.

RESULTS

Demographics

The characteristics of patients, clinical courses, and IMK samples are presented in Table 1.

The 39 kidney transplant recipients consisted of 10 de novo and 29 maintenance-phase patients. The median age was 48 years (range, 22–75 y). Thirteen patients (33.3%) received cadaveric kidney grafts and 26 patients underwent living-donor transplantation. Twenty-nine patients (71.8%) used tacrolimus and 9 (23.1%) used cyclosporine. Two patients (5.1%) did not use a calcineurin inhibitor. Twenty-nine patients (71.8%) received mycophenolate mofetil. Median best serum creatinine during the period was 1.36 mg/dL (range, 0.85–5.60 mg/dL). No patient suffered from acute or chronic rejection. Seven patients (20.5%) contracted viral infections. The median postoperative period was 384 days (range, 6–11,563 days), and a median of 6 samples were collected from each patient.

Factors Influencing IMK Values in Kidney Transplants

Multiple regression analysis of factors influencing IMK values in kidney transplant patients is summarized in Table 2. For the univariate analysis, white blood cell,

Table 1. Patient Characteristics, Clinical Course, and IMK Samples

Patients	
No of patients	39
De novo	10 (25.6%)
Maintenance phase	29 (74.4%)
Post-Tx period, median	1,546 (125–11,535) d
Age, median	48 (22–75) y
Gender	
Male	27 (69.2%)
Female	12 (30.8%)
Type of transplant	
Deceased donor	13 (33.3%)
Living donor	26 (66.7%)
Number of transplant	
1st	37 (94.9%)
2nd	2 (5.1%)
Immunosuppression regimen	
Tac + MMF + MP	23 (59.0%)
Tac + MZ + MP	5 (12.8%)
CsA + MMF + MP	6 (15.4%)
CsA + MZ + MP	3 (7.7%)
AZ or MZ + MP	2 (5.1%)
Clinical course	
Best Cre during the study, median	1.36 (0.85–5.60) mg/dL
Rejection	0 (0.0%)
Viral infection	7 (20.5%)
CMV (except for primary infection)	2 (5.1%)
BKV	3 (7.7%)
VZV (shingles)	2 (5.1%)
HSV	1 (2.6%)
IMK samples	
No of tests	224
Post-Tx period, median	384 (6–11,563) d
No of times/patient, median	6 (1–11)

Abbreviations: IMK, Immuknow; Tx, transplantation; Tac, tacrolimus; MMF, mycophenolate mofetil; MP, methylprednisolone; MZ, mizoribine; CsA, cyclosporine; AZ, azathioprine; Cre, creatinine; CMV, cytomegalovirus; BKV, BK virus; VZV, varicella zoster virus; HSV, herpes simplex virus.

Table 2. Multiple Regression Analysis of Factors Influencing IMK Value in Kidney Transplants

	Univariate analysis		Multivariate analysis	
Factor	β	*P* value	β	*P* value
Characteristics				
Age (y)	−0.303	<.001	−0.023	.67
Gender (male vs female)	0.021	.76	–	–
Post-transplant days	−0.108	.11	–	–
Tac use (yes vs no)	0.156	.02	0.180	.42
MMF use (yes vs no)	0.021	.76	–	–
Laboratory data				
WBC (/μL)	0.757	<.001	0.364	.03
Neutrophils (/μL)	0.714	<.001	0.021	.02
Lymphocytes (/μL)	0.125	.07	–	–
Monocytes (/μL)	0.424	<.001	−0.008	.90
CRP (mg/mL)	0.164	.01	−0.014	.78

Abbreviations: IMK, Immuknow; MMF, mycophenolate mofetil; WBC, white blood cells; CRP, C-reactive protein.

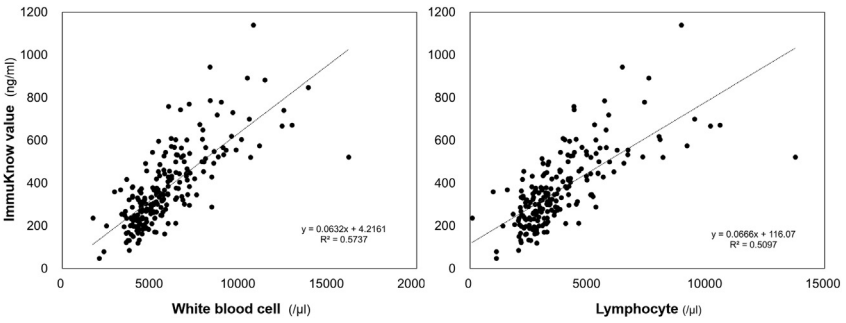

Fig 1. Correlation of Immuknow value versus the numbers of white blood cells and lymphocytes.

neutrophil, and monocyte counts and CRP value positively correlated, and age negatively correlated, with IMK value. Tacrolimus users had lower IMK values. Multivariate analysis revealed that only 2 factors, white blood cell and neutrophil counts, positively correlated with IMK value ($P = .03$ and $P = .02$, respectively). The correlation coefficients (R^2) between IMK value and white blood cell and neutrophil counts were 0.573 and 0.510, respectively (Fig. 1).

Relationship Between Viral Infection and IMK Value

Patient details according to viral infection are described in Table 3, and the relationship between postoperative days and IMK value classified by viral infection is depicted in Figure 2.

Eight patients developed viral infections, including CMV ($n = 2$), BKV ($n = 3$), shingles ($n = 2$), and HSV ($n = 1$) infections. Five occurred in the early period (<50 days) and 3 in the late period (>120 days) after transplantation. The IMK levels in patients who developed an infection in the early period were within normal limits; however, those in the late period were significantly lower than 200 ng/mL (421.0 ± 162.6 vs 153.7 ± 32.7; $P = .02$).

DISCUSSION

Although immunosuppression therapy is essential for transplant recipients to avoid rejection, over-immunosuppression leads to infections that affect patient morbidity and mortality. Until recently, there have been few methods available to directly measure total immune activity.

Approved by the US Food and Drug Administration in April 2002, the Cylex Immuknow assay has been shown to be capable of measuring the global immune response in transplant patients [2]. The clinical validity of the Immuknow assay as an objective tool for assessing immune function has been previously demonstrated in a report that showed that a recipient with a low IMK value was more likely to develop an infection [10]. Our research group reported that 6 of 13 liver transplant recipients with low IMK values (<225 ng/mL) suffered from infectious complications [11]. However, more recently, Xiaoting et al showed that the IMK assay was not able to identify the risk of infection in organ transplant recipients [12].

Among renal transplant recipients, the validity of the IMK assay is more controversial for particular virus infections. Bennett et al reported that the IMK assay was not useful in identifying BKV-infected patients [8]. Huskey et al reported that low IMK values did not correlate with episodes of future opportunistic infection [7]. However, these studies measured the entire post-transplantation period. The present study also did not identify differences between viral-infected and -noninfected patients in all periods (data not shown). However, Gralla et al reported that although low IMK values in the early post-transplantation period are not associated with BK virus infection, after 6 months they were able to identify the risk for BK virus infection [5]. These data are consistent with our findings.

Table 3. Details of Patients with Viral Infection

No	POD	IMK	WBC	Sex/age	Viral infection	Immunosupression regimen/modification
1	34	286	5,560	M/39	BKV viremia	Tac + MMF + MP/conversion MMF to MZ
2	34	604	10,110	F/41	CMV antigenemia	Tac + MMF + MP/none
3	41	330	4,640	F/50	BKV viruria	Tac + MMF + MP/conversion MMF to MZ
4	41	572	6,150	M/45	BKV nephropathy	Tac + MMF + MP/discontinuation of FK, MMF
5	48	268	3,550	M/60	CMV antigenemia	Tac + MMF + MP/none
6	524	188	4,710	F/48	Shingles	Tac + MMF + MP/none
7	881	150	3,990	M/41	HSV angular cheilitis	Tac + MMF + MP/none
8	4024	123	3,600	M/75	Shingles	CsA + MMF + MP/reduction of MMF

Abbreviations: POD, postoperative days; IMK, Immuknow value; WBC, white blood cell count/μL; BKV, BK virus; CMV, cytomegalovirus; Tac, tacrolimus; MZ, mizoribine; MMF, mycophenolate mofetil; MP, methylprednisolone; CsA, cyclosporine.

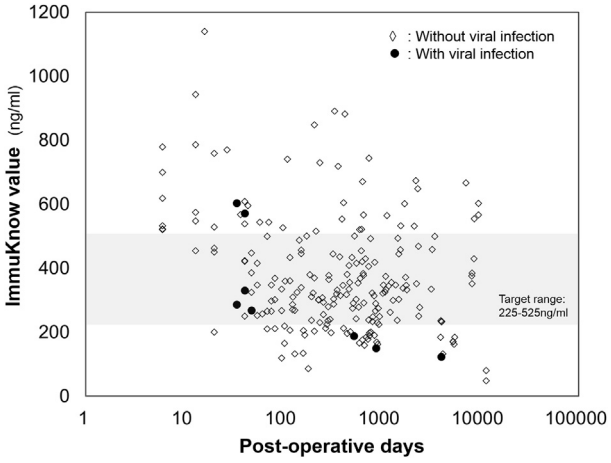

Fig 2. The relationship of postoperative days versus IMK value classified by viral infection.

We further identified a strong correlation with IMK value and white blood cell or neutrophil count. This phenomenon was also reported previously by Serban et al [13]. We also found that IMK values decrease with time after transplantation during the 120-day postoperative period (data not shown). This change in IMK value may be caused by a decrease in white blood cell count. Furthermore, we speculate that the reliability of IMK values in the late post-transplantation period is due to stable white blood cell counts during this period.

There are a number of limitations of the present study. First, it is a retrospective study. Second, it involves heterogeneous factors, such as patient characteristics, post-transplantation period, and immunosuppression regimen. Third, the number of patients is very small, and viral infection episodes are rare.

Recently, Moon et al reported success in curing BKV infection without rejection by adjusting the immunosuppressant according to the patient's IMK value in kidney transplant patients [4]. We are planning a prospective study to adjust immunosuppressant levels in patients with low IMK values during the late post-transplantation period to prevent viral infection, especially BKV.

In conclusion, the IMK assay is a useful test for screening high-risk patients for viral infections during the late post-transplantation period.

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