International Immunopharmacology 75 (2019) 105803

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Assessment of serum Tim-3 and Gal-9 levels in predicting the risk of infection after kidney transplantation

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ARTICLE INFO

Keywords:
Tim-3
Galectin-9
Kidney transplantation
Infection
Prediction

ABSTRACT

Infection remains a major cause of morbidity and mortality after kidney transplantation (KT). Reliable biomarkers to predict post-transplant infection are lacking. We investigated the predictive performance of pre- and post-transplant levels of T-cell immunoglobulin and mucin domain-3 (Tim-3) and Galectin-9 (Gal-9), two pleiotropic immunomodulatory molecules, in early identification of infection. Serum Tim-3 and Gal-9 were paired measured before and 30 days after transplantation (PTD 30) in 95 KT recipients (KTRs). The decline rates of Tim-3 and Gal-9 were calculated relative to pre-transplant levels. KTRs with infection history had significantly higher levels of PTD 30 Tim-3 and Gal-9, and slower decrease rates of Gal-9 compared to non-infected recipients, while no difference was observed between two groups regarding pre-transplant levels. The AUCs for predicting 1-year post-transplant infection were 0.653 and 0.711 for post-transplant Tim-3 and Gal-9, 0.664 and 0.670 for relative Tim-3 and Gal-9, respectively. After adjusting for potential confounders, PTD 30 Tim-3, Gal-9 and relative Gal-9 remained as independent risk factors for post-transplant infection. Our results suggested that PTD 30 Tim-3 and Gal-9 and relative decrease of Gal-9 were promising predictors for identifying KTRs with high risk of infection, while pre-transplant Tim-3 and Gal-9 showed no predictive power to infection.

1. Introduction

Over the past few decades, clinical physicians have been working on finding optimal immunosuppressive therapy that could set kidney transplant recipients (KTRs) free from rejection without suffering from overimmunosuppression, such as infection and malignancy. However, infection remains one of the most common complications in KTRs with non-negligible morbidity and mortality in these years [1]. Aside from directly causing death, it was also reported to be associated with decreased graft survival [2–4]. Usually, immunosuppressant concentrations are routinely monitored to estimate the degree of immunosuppression; nevertheless, such method is not sufficient to reflect the intrinsic risk of infection in KTRs. Therefore, a growing number of studies [5–8] have focused on immune-related biomarkers that may serve as direct indicators for impending infection after transplantation, and may assist in minimizing deleterious consequences of infection through appropriate prophylaxis and timely adjustment of immunosuppressive therapy.

In recent years, T-cell immunoglobulin and mucin domain-3 (Tim-3) and its best-known ligand galectin-9 (Gal-9) have emerged as pleiotropic immune regulators that involve in the negative regulation of T cells responses and the induction of immune tolerance [9]. As a type I transmembrane protein, Tim-3 expresses on multiple immune cells such as activated or exhausted T cells [10], natural killer (NK) cells [11] and other innate and adaptive immune cells [12]. Early animal experiment studies elucidated that the interaction between cellular Tim-3 and Gal-9 would result in the suppression of Th1 and Th17 cellular immune responses and the promotion of regulatory T cells (Tregs) differentiation [13,14]. Later, such immune alteration was proven to be critical in maintaining the immune tolerance of solid organ transplantation (SOT) [15–18]. Blocking Tim-3/Gal-9 interaction with anti-Tim-3 antibody accelerated the graft loss in a chronic cardiac allograft rejection model, which was characterized by increased Th1 and Th17 polarization and inhibited Treg induction [19]. In addition to inhibit T cells responses directly by inducing cell death and exhaustion [14], Tim-3/Gal-9 axis was also established to suppress immune responses indirectly by expanding myeloid-derived suppressor cells (MDSCs), which are inhibitors of T cells responses [20].

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https://doi.org/10.1016/j.intimp.2019.105803
Received 23 February 2019; Received in revised form 2 July 2019; Accepted 27 July 2019
Available online 08 August 2019

As counterparts of cellular Tim-3 and Gal-9, the soluble forms of Tim-3 and Gal-9 in serum, plasma or urine were extensively investigated to explore their potential as biomarkers of T-cell responsiveness in SOT [21–23]. Soluble Tim-3 (sTim-3) was initially identified in mice as an alternatively spliced variant [13] and then Möller-Hackbarth et al. confirmed that it was also cleaved from membrane Tim-3 [24]. Numerous studies have found that higher level of sTim-3 and elevated expression of Gal-9 were significantly associated with acute allograft rejection in KTRs [21,25,26]. In addition, our published data demonstrated that both sTim-3 and sGal-9 were promising biomarkers for chronic allograft dysfunction (CAD) in KTRs [27]. These results indicate that sTim-3 and sGal-9 are important immunoregulatory molecules of alloimmune responses. However, limited studies have concentrated on the association between these soluble markers and post-transplant infection so far [28–31]. In particular, data on their predictive performances for post-transplant infection were deficient. Whether the detection of sTim-3 and sGal-9 could be used to assess the risk of post-transplant infection is still unknown.

Therefore, we dynamically measured the serum levels of Tim-3 and Gal-9 before and 30 days after transplantation (PTD 30) to explore their potential utilities in predicting the occurrence of post-transplant infection in a cohort of 95 KTRs. In addition, the predictive potential of relative changes of Tim-3 and Gal-9 from baseline to PTD 30 for subsequent infection was also investigated in this study.

2. Materials and methods

2.1. Study population and diagnosis of infections

The study was performed on a library of sera from 95 adult KTRs who underwent KT between July 2014 and June 2016 in West China Hospital of Sichuan University. KTRs were included if they met the following criteria: (1) recipient age > 18 years old at transplantation; (2) one-year regular follow-up data were available in our hospital. Recipients with known primary autoimmune diseases (such as systemic lupus erythematosus and systemic sclerosis), chronic infection (such as viral hepatitis or tuberculosis infection) and malignancy were excluded. This study was approved by the Ethics Committee of West China Hospital and written informed consent was obtained from each participant before the enrollment. The study was performed according to the Code of Ethics of the World Medical Association (Declaration of Helsinki).

95 KTRs were divided into two groups according to the occurrence of infection event between the first month and 12 months after transplantation. Recipients who experienced infection that required hospitalization at least once were included in the Infection group, while the control (Non-Infection) group enrolled patients without any infection episode during the follow-up period. All infection episodes were carefully reviewed to document their locations, durations and pathogenic microorganisms. For recipients with multiple episodes of infection, the first occurrence time was defined as the time-to-infection. Urinary tract infection (UTI) was defined as the onset of local urinary irritation symptoms such as urgency, frequency and dysuria in combination with the positive culture result. UTI with negative urine culture was also counted if clinically suggestive and empirically antibiotic therapy was effective. Pulmonary infection was diagnosed on a clinical basis, evaluated by thoracic computer tomography (CT), confirmed with blood or sputum culture and effectiveness of clinical treatment. If one or more of the following criteria were met, pulmonary infection can be determined: (1) a positive culture result of an unambiguous pathogenic microorganism was obtained; (2) clinical data and CT results supported the diagnosis of infection without culture-confirmed pathogens, but symptoms were completely ameliorated with antimicrobial treatment. We considered infection episodes as the occurrence of any clinical event including bacterial, viral, fungal and polymicrobial infections. Bacterial and fungal infection included both culture-proven and probable episodes. Probable episodes were identified by serology, antigen assays and suggestive clinical symptoms with prompt response to empirically antibiotic or antifungal therapies in the absence of microbiological records. Viral infection was determined with serological and molecular DNA-based methods. Cytomegalovirus (CMV) infection was primarily diagnosed by the presence of either anti-CMV IgM (> 22 AU/ml) or IgG (> 14 U/ml) in symptomatic patients, then it was confirmed by using quantitative nucleic acid amplification testing (QNAT) to quantify CMV-DNA in blood sample. The diagnosis of herpes simplex virus (HSV) infection or varicella zoster virus (VZV) infection were based on the appearance of unilateral vesicular lesions and confirmed by a dermatologist. The diagnostic tests for BK virus infection were viral load assessment and searching of decoy cells in urine sample as well as pathological biopsy of allograft.

2.2. Immunosuppression and prophylaxis regimens

Maintenance immunosuppression consisted of tacrolimus, mycophenolate mofetil and steroids. Tacrolimus was initiated on the day of surgery at a daily dose of 2.0 mg or 3.0 mg and adjusted to target trough level of 5-8 ng/ml. Mycophenolate mofetil was administered at 2.0 g/day on the day of surgery and adjusted to target area under the curve (AUC) values between 45 and 75 mg*h/l. Steroid treatment was given intravenously (IV) with 1000 mg methylprednisolone at the time of transplantation, and then intravenous 500 mg daily for next 2 days. Thereafter, the regimen was followed by 60 mg/day of oral prednisone, gradually tapering to a maintenance dose of 5 or 10 mg daily after 2 weeks.

All recipients received a preoperative single dose of IV cefmetazole as a prophylaxis against intraoperative infection. Trimethoprim-sulfamethoxazole (480 mg daily) was administered, for 6 months, as the prophylaxis for *Pneumocystis jiroveci pneumonia*. In patients with CMV seropositive or at high risk of CMV disease (serologic mismatch: donor +/recipient-), long term prophylaxis was performed with oral valganciclovir hydrochloride (900 mg daily, with dose adjusted for renal function) or ganciclovir (1000 mg 3 times daily, with dose adjusted for renal function) for 3 months.

2.3. Biomarker analysis

Blood samples presenting paired time points of pre-transplantation and PTD 30 were collected from 95 KTRs, among whom 17 recipients only had one sample. Serum samples were obtained after centrifugation and stored in three aliquots at −80 °C until the detection of Tim-3 and Gal-9. Serum concentration of Tim-3 was quantified using enzyme-linked immunosorbent assay (ELISA), strictly according to the manufacturer's instructions. Serum level of Gal-9 was determined by Bio-Plex® suspension array system (Bio-Rad Laboratories Inc., Hercules, CA, USA). Both Human Tim-3 Quantikine ELISA Kit and Human Premixed Multi-Analyte Kit were purchased from R&D Systems (Minneapolis, MN, USA).

2.4. Statistical analysis

Statistical analyses were completed with SPSS software (version 23.0, SPSS Inc., Chicago, IL, USA). Graphs were generated with GraphPad Prism (Version 6.0, Graphpad, La Jolla, CA, USA). Data were demonstrated as absolute number, mean ± standard deviation or median (interquartile range) according to the data type. Chi-square or Fisher exact tests were utilized to compare categorical variables between groups. Student's *t*-test or Mann-Whitney *U* test was applied to compare continuous variables with normal distribution and skewed distribution, respectively. Following formula was used to calculate the relative change rates of biomarkers: Relative change rates (%) = (PTD 30 − pre-transplant) / pre-transplant level of biomarker * 100%. Receiver-operating characteristic (ROC) curve was conducted to assess

Table 1
Summary of baseline and clinical characteristics in kidney transplant recipients and donors.

	Overall	Non-Infection group	Infection group	*P* value[a]
Recipient characteristics				
Number	95	63	32	/
Age (yr)	28 (25–34)	28 (25–34)	29 (26–34)	0.503
Gender (M/F)	71/24	46/17	25/7	0.628
BMI (kg/m^2)	20.3 (18.9–22.2)	20.0 (18.8–22.2)	20.9 (19.0–22.2)	0.402
Pre-transplant urine output (ml/day)	200 (50–575)	200 (50–550)	250 (0–600)	0.721
Pre-transplant dialysis type				
Hemodialysis	80	54	26	
Perioneal dialysis	3	2	1	0.822
No dialysis	12	7	5	
Dialysis vintage (months)	12 (6–24)	12 (6–12)	12 (9–36)	0.085
Pre-PRA (%)	2.2 ± 0.7	1.7 ± 0.6	3.2 ± 1.8	0.332
Induction therapy				
Anti-CD25	76	51	25	
Anti-thymoglobulin antibodies	13	10	3	0.165
No induction	6	2	4	
HLA mismatches (A, B, DR, DQ)	4 (3–4)	4 (4–4)	4 (3–4)	0.282
Delayed graft function (Y/N)	5/90	3/60	2/30	0.759
Acute graft rejection (Y/N)[b]	13/82	6/57	7/25	0.098
Trough concentration of tacolimus (ng/ml)[b]	5.6 ± 0.3	5.5 ± 0.2	5.9 ± 0.7	0.470
Donor characteristics				
Age (yr)	49 (43–52)	48 (42–52)	51 (35–54)	0.185
Gender (M/F)	30/64	22/41	8/23	0.373
Donor type				
Living-related donor	88	58	30	0.766
Living–unrelated donor[c]	7	5	2	
Cold ischaemia time (min)	113.3 ± 7.1	115.0 ± 10.3	110.6 ± 9.4	0.771
Baseline serostatus				
CMV status D+/R+	88 (92.6%)	60 (95.2%)	28 (87.5%)	
CMV status D+/R-	3 (3.2%)	1 (1.6%)	2 (6.3%)	0.557
CMV status $D_{unknown}$/R+	2 (2.1%)	1 (1.6%)	1 (3.1%)	
CMV status $D_{unknown}$/$R_{unknown}$	2 (2.1%)	1 (1.6%)	1 (3.1%)	

PRA, panel reactive antibody; HLA, human leucocyte antigen;
[a] Recipients without infection event versus recipients with infection episodes.
[b] The acute graft rejection and trough concentration of tacrolimus were obtained prior to the truncated date.
[c] Living-unrelated donor: spouses.

the predictive performances of biomarkers and the optimal cut-off value was determined by maximum value of Youden-J indexes (sensitivity + specificity −1). Univariate and multivariate (backward conditional selection) Cox proportional hazard regression models were used to weigh the impact of the potential risk factors for post-transplant infection. Some clinically relevant parameters were forced into the models irrespective of their univariate analysis results. A two-tailed *P* value < 0.05 was considered statistically significant in all tests.

3. Results

3.1. Baseline characteristics and post-transplant infections

The demographic and clinical data of recipients and donors were summarized in Table 1. Of 95 KTRs, 63 recipients (Non-Infection group, 66.3%) were completely free from infection and 32 patients (Infection group, 33.7%) had experienced at least one episode of infection during the first 12 months after transplantation. No significant differences were observed between Non-Infection and Infection groups in terms of recipient and donor baseline characteristics, pre-transplant treatment, operation-related data and post-transplant complications. All 95 recipients maintained on tacrolimus-based triple immunosuppression treatment (tacrolimus + mycophenolate mofetil + steroids). And no significant difference was observed in trough concentrations of tacrolimus prior to the truncated date. Characteristics of infections were presented in Table 2. Bacterial (43.7%) and polymicrobial infection (31.3%) were the common causes of infection, and the majority of infections occurred in the lungs (81.3%). Half of the infected KTRs (50%) developed infection during the early period (months 1–6), while the rest developed infection during the late period (months 7–12).

Table 2
Characteristics of the infections occurring between 1 and 12 months after transplantation.

	N	(%)
Microbiological identification		
Bacterial infection	14	(43.7)
Viral infection	5	(15.6)
Fungal infection	3	(9.4)
Polymicrobial infection	10	(31.3)
Locations of infection		
Lungs	26	(81.3)
Urinary tract	1	(3.1)
Other	2	(6.2)
Multiple sites	3	(9.4)
Initial time of infection		
1–3 months	9	(28.1)
4–6 months	7	(21.9)
7–9 months	6	(18.8)
10–12 months	10	(31.2)

3.2. Levels of serum Tim-3 and Gal-9 at pre-transplant and PTD 30 in Non-Infection and Infection groups

No significant differences were found between Non-Infection and Infection groups with regard to the levels of pre-transplant Tim-3 and Gal-9. While the levels of Tim-3 (median: 467.2 vs. 409.1 pg/ml; $P = 0.026$) and Gal-9 (median: 7009.1 vs.4867.4 pg/ml; $P = 0.004$) at PTD 30 were significantly higher in KTRs who developed infection

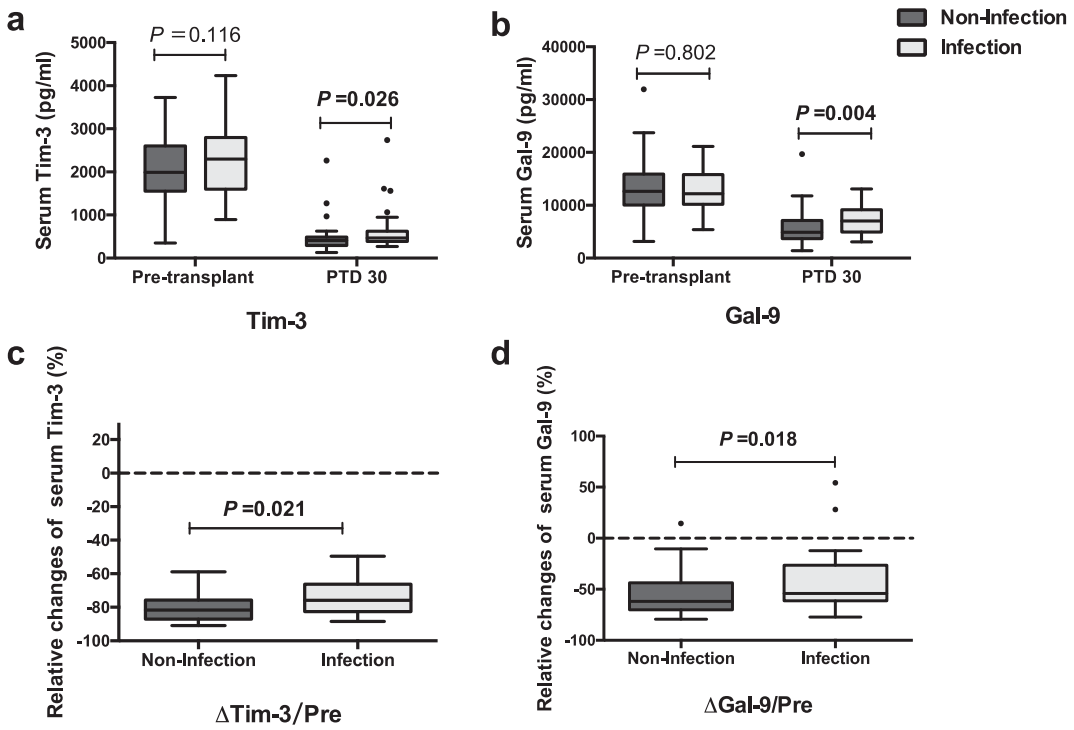


Fig. 1. Serum levels of Tim-3 and Gal-9, relative changes of Tim-3 and Gal-9 at pre-transplant and PTD 30 time points in Non-Infection and Infection groups. a–b. Levels of Tim-3 and Gal-9 before and 30 days after transplantation; c–d. Changes of serum Tim-3 and Gal-9 relative to pre-transplant levels. PTD: post-transplant days.

compared to those infection-free KTRs (Fig. 1a and b). In general, serum Tim-3 and Gal-9 decreased dramatically from pre-transplant measurement to PTD 30 in majority of KTRs. The relative decline rates of serum Tim-3 [Non-Infection vs. Infection (median): −81.6% vs. −74.9%; $P = 0.021$] and serum Gal-9 [Non-Infection vs. Infection (median): −62.0% vs. −54.1%; $P = 0.018$] were significantly higher in Non-Infection group compared to Infection group (Fig. 1c and d).

3.3. Predictive performances of serum Tim-3 and Gal-9 for post-transplant infection in KTRs

To identify the predictive performances and the optimal cut-off values of PTD 30 Tim-3, PTD 30 Gal-9 and the relative changes of Tim-3 and Gal-9 for predicting post-transplant infection event, we performed the ROC curve analyses. As shown in Fig. 2, the calculated AUC for PTD 30 Tim-3 was 0.653 (95% confidence interval, 95%CI 0.531–0.775) with a sensitivity of 67.9% and a specificity of 60.0% at the optimal cut-off value of 423.1 pg/ml. The AUC for PTD 30 Gal-9 was 0.711 (95%CI 0.592–0.829) with a sensitivity of 75.0% and a specificity of 60.0% at the cut-off value of 5263.1 pg/ml. The AUCs for the relative decline rates of Tim-3 and Gal-9 were 0.664 and 0.670, respectively. Given the cut-off of −79.7% (for Tim-3) and −59.8% (for Gal-9), relative Tim-3 could predict infection with a sensitivity of 68.0% and a specificity of 57.2% and relative Gal-9 with a sensitivity of 72.0% and a specificity of 59.2%.

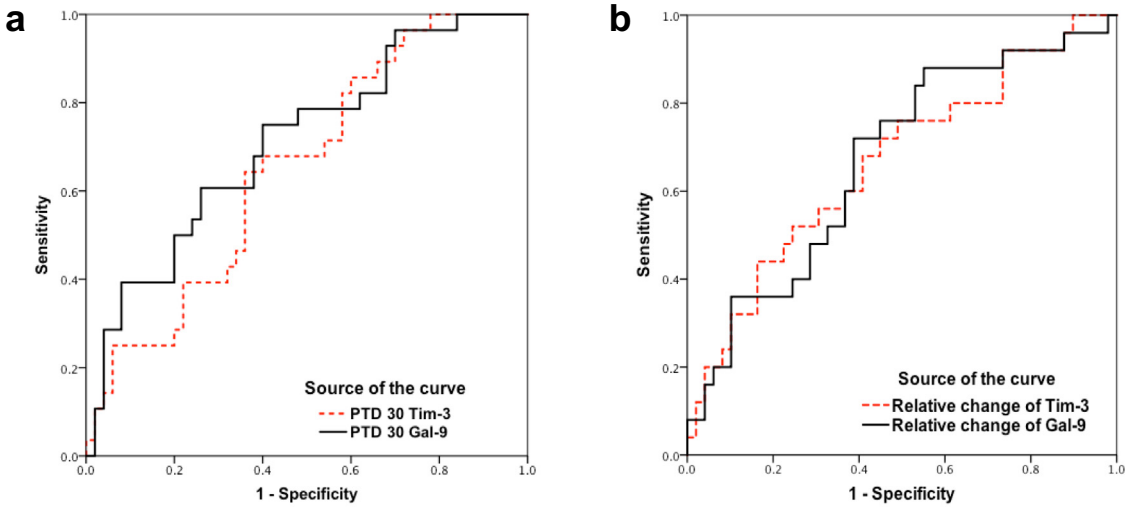
3.4. Univariate and multivariate analyses of four markers as prognostic factors for post-transplant infection

PTD 30 Tim-3, PTD 30 Gal-9, relative change of Tim-3 and relative change of Gal-9 were respectively adjusted for clinically relevant factors, including recipient age, donor age, induction therapy, acute graft rejection and trough concentration of tacrolimus by using multivariate analysis. After adjusting for potential confounders, PTD 30 Tim-3 levels > 423.1 pg/ml (HR 2.713, 95%CI 1.294–5.690; $P = 0.008$), PTD 30 Gal-9 levels > 5263.1 pg/ml (HR 2.510, 95%CI 1.166–5.403; $P = 0.019$) and relative change of Gal-9 > −59.8% (HR 2.087, 95%CI 1.003–4.343; $P = 0.049$) remained as significant, independent predictors of subsequent infection (Table 3).

4. Discussion

To our best knowledge, this is the first study investigated the predictive potential of serum Tim-3 and Gal-9 at different time points (pre-transplantation and PTD 30) to predict the risk of infection in KTRs within 12 months after transplantation. The data indicated that pre-transplant levels of Tim-3 and Gal-9 were not associated with the occurrence of impending infection, while higher concentrations of Tim-3 and Gal-9 at 30 days after transplantation were significant risk factors of infection in KTRs. Additionally, the rapid decline rates of Gal-9 relative to pre-transplant levels were also found to be protective factors of post-transplant infection.

Monitoring pre-transplant immune molecules appeared to be a promising way for predicting short- or long-term outcomes such as acute rejection [32–34] and infection [8,35] after transplantation, as evidenced by a series of researches. However, we only observed that serum levels of Tim-3 and Gal-9 in patients with end-stage renal diseases (ESRD) were much higher than those after transplantation, no additional predictive power of these two pre-transplant biomarkers for post-transplant infection was found in this study. This might be related to the great differences in immunomodulatory states between ESRD and post-transplantation status. In the ESRD stage, one of the striking feature of uraemic immune dysregulation is the increased activation-induced T cell apoptosis and reduced effector T cell responses, which may promote the up-regulation of co-inhibitory molecules such as Tim-3 and Gal-9 and then contribute to an increasing susceptibility to infections in patients with ESRD [36–38]. Whereas, in KTRs, uraemia-associated immune disorders would be considerably restored after successful transplantation and immunosuppressant therapies. So, the predictive abilities of pre-transplant Tim-3 and Gal-9 levels for post-transplant



Markers	AUC (95% CI)	Cut-off	Sensitivity	Specificity	PPV	NPV	PLR	NLR
PTD 30 Tim-3	0.653 (0.531-0.775)	423.1	67.9%	60.0%	46.8%	79.2%	1.70	0.54
PTD 30 Gal-9	0.711 (0.592-0.829)	5263.1	75.0%	60.0%	49.0%	82.6%	1.88	0.42
Relative change of Tim-3	0.664 (0.532-0.797)	-79.7%	68.0%	57.2%	44.9%	72.3%	1.59	0.56
Relative change of Gal-9	0.670 (0.540-0.800)	-59.8%	72.0%	59.2%	46.9%	80.4%	1.76	0.47

Fig. 2. Predictive performances of PTD 30 Tim-3, PTD 30 Gal-9 and relative changes of Tim-3 and Gal-9 for the prediction of infection occurred between month 1 and month12 after transplantation. a. ROC curves for PTD 30 Tim-3 and Gal-9; b. ROC curves for relative changes of Tim-3 and Gal-9. The table below shows the predictive values of each marker.

Table 3
Univariate and multivariate Cox proportional hazard regression analyses for the risk of infection within 12 months after transplantation.

Variables	Univariate analysis			Multivariate analysis		
	HR	95%CI	*P*	HR	95%CI	*P*
Recipient age	1.020	0.982–1.059	0.312	–	–	–
Donor age	1.019	0.980–1.060	0.337	–	–	–
Induction therapy	0.758	0.440–1.306	0.318	–	–	–
Acute graft rejection	1.791	0.774–4.144	0.173	–	–	–
Trough concentration of tacrolimus[a]	1.029	0.913–1.160	0.637	–	–	–
PTD 30 Tim-3 > 423.1 pg/ml[b]	2.792	1.376–5.663	0.004	2.713	1.294–5.690	0.008
PTD 30 Gal-9 > 5263.1 pg/ml[b]	2.529	1.218–5.250	0.013	2.510	1.166–5.403	0.019
Relative change of Tim-3 > −79.7%[b]	2.100	1.047-4.209	0.037	1.997	0.963–4.140	0.063
Relative change of Gal-9 > −59.8%[b]	2.172	1.079-4.374	0.030	2.087	1.003–4.343	0.049

HR, hazard ratio; CI, confidence interval.
[a] The trough concentration of tacrolimus obtained prior to the truncated date.
[b] PTD 30 Tim-3, PTD 30 Gal-9, Relative change of Tim-3 and Relative change of Gal-9 were respectively adjusted for recipient age, donor age, induction therapy, acute graft rejection and trough concentration of tacrolimus. The adjusted HRs are presented in multivariate analysis.

infection were likely to be weakened.

In the current study, both PTD 30 Tim-3 and Gal-9 were significantly higher in patients with subsequent infection than those without. Further ROC analyses demonstrated that PTD 30 Gal-9 had good ability to predict infection occurred during first 12 months after KT, as demonstrated by the AUC of 0.711. While the capability of PTD 30 Tim-3 to differentiate two groups was inferior, with an AUC of 0.653. Importantly, the predictive performances of these two markers remained in multivariate Cox proportional hazard regression analyses after adjusting for potential confounders (Table 3), which confirmed the potential utilities of these biomarkers to aid identification of patients at higher risk of infection prior to an infection episode. The exact mechanisms for these interesting findings are unclear so far. Two potential explanations might be responsible for this phenomenon, which include (i) the incomplete recovery from immune dysfunction in ESRD stage after KT, and (ii) sTim-3 and sGal-9 may act as decoys to block cellular Tim-3/Gal-9 pathway and affect transmission of downstream signals. Many researchers believed that some KTRs might experience irreversible immune dysfunction even after achieving completely recovery of renal transplant function, which would contribute to the occurrence of post-transplant complications such as malignancy, infection and rejection [36]. So, increasing studies were concerning about the early monitoring of post-transplant immune-related biomarkers to predict the undesirable complications after transplantation. To predict infection in KTRs, Fernandez-Ruiz M and his colleagues dynamically analyzed the roles of peripheral blood lymphocyte subsets in 304 KTRs for predicting opportunistic infection (OI). The results showed that $CD8^+$ T cells counts at months 1 and 6 post-transplantation as well as

$CD4^+$ T cell count at month 1 were strongly associated with the development of OI in KTRs without or with Anti-thyroglobulin antibodies (ATG) induction, respectively [5]. Afterwards, serum CD30, a molecule determining the physiological Th1/Th2 balance, was consecutively measured at post-transplant months 1 and 6 in 100 KTRs by the same research group, and the results demonstrated that PTD 30 serum CD30 was a risk factor for post-transplant bacterial infection [8]. In our study, similar results were observed in analyzing the association between PTD 30 Tim-3, Gal-9 and post-transplant infection, which further supported that the detection of post-transplant immune regulators was an effective method to find the promising predictive biomarkers for subsequent infection. In addition, Pushpa Jayaraman's group has found that the binding of Th1 cell surface molecule Tim-3 and Gal-9, a ligand expressed on infected macrophages, would lead to the activation of macrophages and stimulate bactericidal activity [39]. Higher levels of sTim-3 and sGal-9 may interfere with cellular Tim-3/Gal-9 interaction, affecting the activity of anti-infection. Moller-Hackbarth K et al found that inhibiting the shedding of Tim-3 (sTim-3) from LPS-induced monocytes would upregulate the secretion of IL-12 and TNF- α, two cytokines that are important in fighting against infection [24]. In line with this, F. Ren et al clinically observed that sTim-3 showed negative correlations with IL-12 and TNF- α in patients with sepsis [40]. All these results indicated that sTim-3 played a harmful role in anti-infections. Combined our results with above findings, we speculated that higher levels of PTD 30 sTim-3 and sGal-9 would increase the susceptibility to infection probably through downregulating the expression of some anti-inflammatory cytokines, such as IL-12 and TNF- α.

It was evident that the levels of soluble Tim-3 and Gal-9 were dropped dramatically from pre-transplant to PTD 30 after transplantation in this study. Calculating the decline rates of Tim-3 and Gal-9 relative to pre-transplant levels may provide information about the immunological recovery extents following transplantation and immunosuppression, and this might be helpful in forecasting post-transplant infection. Our results demonstrated that the relative decline rates of Tim-3 and Gal-9 were significantly greater in Non-Infection group compared to Infection group. Univariate and multivariate analyses results revealed that rapid decrease rate of Gal-9 was independently protective factor for post-transplant infection, while relative change of Tim-3 could not forecast the development of infection after transplantation when adjusted for other variables. These results suggested the superiority of dynamically monitoring of Gal-9 than Tim-3 in terms of predicting infection complication in KTRs.

There are several limitations in this study. Firstly, it was a single-center study with limited sample size; the results should be taken with caution. Secondly, some infection events were diagnosed without culture results of pathogens, we could not figure out the associations between Tim-3, Gal-9 and particular types of infection. Thirdly, only one post-transplant point sample was available in our study, so whether dynamically monitoring of serum Tim-3 and Gal-9 at specific time points after transplantation would be more valuable in predicting infection was unclear.

In conclusion, our results indicate that higher levels of PTD 30 Tim-3 and Gal-9 were novel and potentially useful predictors for infection occurred within 12 months after transplantation. While elevated levels of pre-transplant Tim-3 and Gal-9 were not functional molecules of high risk of post-transplant infection. In addition, the relative decrease rate of Gal-9, not Tim-3, showed the ability to identify the KTRs with high risk of infection. Future replications of these results in larger prospective studies (multi-center if possible) are required to validate their significances.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

This study was supported by grants from the National Natural Science Foundation of China [grant numbers: 81771714, 81571561, 81871713].

References

[1] J.A. Fishman, Introduction: infection in solid organ transplant recipients, Am. J. Transplant. 9 (2009) S3–S6.
[2] M.N. Al-Hasan, R.R. Razonable, W.K. Kremers, L.M. Baddour, Impact of Gram-negative bloodstream infection on long-term allograft survival after kidney transplantation, Transplantation 91 (11) (2011) 1206–1210.
[3] E.C. Lorenz, F.G. Cosio, The impact of urinary tract infections in renal transplant recipients, Kidney Int. 78 (8) (2010) 719–721.
[4] P.J. Dupont, O. Manuel, M. Pascual, Infection and chronic allograft dysfunction, Kidney Int. Suppl. (119) (2010) S47–S53.
[5] M. Fernandez-Ruiz, F. Lopez-Medrano, L.M. Allende, A. Andres, A. Garcia-Reyne, C. Lumbreras, R. San-Juan, J.M. Morales, E. Paz-Artal, J.M. Aguado, Kinetics of peripheral blood lymphocyte subpopulations predicts the occurrence of opportunistic infection after kidney transplantation, Transpl. Int. 27 (7) (2014) 674–685.
[6] M. Fernández-Ruiz, F. López-Medrano, P. Varela-Peña, D. Lora-Pablos, A. García-Reyne, E. González, J.M. Morales, R. San Juan, C. Lumbreras, E. Paz-Artal, et al., Monitoring of immunoglobulin levels identifies kidney transplant recipients at high risk of infection, Am. J. Transplant. 12 (10) (2012) 2763–2773.
[7] T. Crepin, E. Gaiffe, C. Courivaud, C. Roubiou, C. Laheurte, B. Moulin, L. Frimat, P. Rieu, C. Mousson, A. Durrbach, et al., Pre-transplant end-stage renal disease-related immune risk profile in kidney transplant recipients predicts post-transplant infections, Transpl. Infect. Dis. 18 (3) (2016) 415–422.
[8] M. Fernandez-Ruiz, P. Parra, F. Lopez-Medrano, T. Ruiz-Merlo, E. Gonzalez, N. Polanco, J. Origuen, R. San Juan, A. Andres, J.M. Aguado, Serum sCD30: a promising biomarker for predicting the risk of bacterial infection after kidney transplantation, Transpl. Infect. Dis. 19 (2) (2017).
[9] K. Sakuishi, P. Jayaraman, S.M. Behar, A.C. Anderson, V.K. Kuchroo, Emerging Tim-3 functions in antimicrobial and tumor immunity, Trends Immunol. 32 (8) (2011) 345–349.
[10] W.D. Hastings, D.E. Anderson, N. Kassam, K. Koguchi, E.A. Greenfield, S.C. Kent, X.X. Zheng, T.B. Strom, D.A. Hafler, V.K. Kuchroo, TIM-3 is expressed on activated human CD4+ T cells and regulates Th1 and Th17 cytokines, Eur. J. Immunol. 39 (9) (2009) 2492–2501.
[11] N.H. Ying Ju, J. Meng, X. Wang, X. Zhang, Z. Di, Y. Liu, F. Zhu, L. Zhang, W. Sun, X. Liang, L. Gao, C. Ma, T cell immunoglobulin- and mucin-domain-containing molecule-3 (Tim-3) mediates natural killer cell suppression in chronic hepatitis B, J. Hepatol. 52 (11) (2010) 322–329.
[12] A.C. Anderson DEA, L. Bregoli, W.D. Hastings, N. Kassam, C. Lei, R. Chandwaskar, J. Karman, W.S. Ee, M. Hirashima, J.N. Bruce LPK, V.K. Kuchroo, D.A. Hafler, Promotion of tissue inflammation by the immune receptor Tim-3 expressed on innate immune cells, Science 318 (2007) 1141–1143.
[13] C.A. Sabatos, S. Chakravarti, E. Cha, A. Schubart, A. Sánchez-Fueyo, X.X. Zheng, A.J. Coyle, T.B. Strom, G.J. Freeman, V.K. Kuchroo, Interaction of Tim-3 and Tim-3 ligand regulates T helper type 1 responses and induction of peripheral tolerance, Nat. Immunol. 4 (11) (2003) 1102–1110.
[14] C. Zhu, A.C. Anderson, A. Schubart, H. Xiong, J. Imitola, S.J. Khoury, X.X. Zheng, T.B. Strom, V.K. Kuchroo, The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity, Nat. Immunol. 6 (12) (2005) 1245–1252.
[15] S. Gupta, T.B. Thornley, W. Gao, R. Larocca, L.A. Turka, V.K. Kuchroo, T.B. Strom, Allograft rejection is restrained by short-lived TIM-3 + PD-1 + Foxp3 + Tregs, J. Clin. Invest. 122 (7) (2012) 2395–2404.
[16] M. Shimmura-Tomita, M. Wang, H. Taniguchi, H. Akiba, H. Yagita, J. Hori, Galectin-9-mediated protection from allo-specific T cells as a mechanism of immune privilege of corneal allografts, PLoS One 8 (5) (2013) e63620.
[17] F. Wang, W. He, J. Yuan, K. Wu, H. Zhou, W. Zhang, Z.K. Chen, Activation of Tim-3-Galectin-9 pathway improves survival of fully allogeneic skin grafts, Transpl. Immunol. 19 (1) (2008) 12–19.
[18] Y. Li, Y. Shi, Z. Huang, Y. Bai, Q. Niu, B. Cai, L. Wang, W. Feng, CNI induced Th17/Treg imbalance and susceptibility to renal dysfunction in renal transplantation, Int. Immunopharmacol. 11 (12) (2011) 2033–2038.
[19] O. Boenisch, F. D'Addio, T. Watanabe, W. Elyaman, C.N. Magee, M.Y. Yeung, R.F. Padera, S.J. Rodig, T. Murayama, K. Tanaka, et al., TIM-3: a novel regulatory molecule of alloimmune activation, J. Immunol. 185 (10) (2010) 5806–5819.
[20] V. Dardalhon, A.C. Anderson, J. Karman, L. Apetoh, R. Chandwaskar, D.H. Lee, M. Cornejo, N. Nishi, A. Yamauchi, F.J. Quintana, et al., Tim-3/galectin-9 pathway: regulation of Th1 immunity through promotion of CD11b + Ly-6G + myeloid cells, J. Immunol. 185 (3) (2010) 1383–1392.
[21] S.K. Shahbaz, F. Pourrezagholi, M. Barabadi, F. Foroughi, M. Hosseinzadeh, P. Ahmadpoor, M. Nafar, M.S. Yekaninejad, A. Amirzargar, High expression of TIM-3 and KIM-1 in blood and urine of renal allograft rejection patients, Transpl. Immunol. 43-44 (2017) 11–20.
[22] P.G. Renesto, V.C. Ponciano, M.A. Cenedeze, N.O. Saraiva Camara, A. Pacheco-Silva, High expression of Tim-3 mRNA in urinary cells from kidney transplant recipients with acute rejection, Am. J. Transplant. Off. J. Am. Soc. Transplant. Am. Soc. Transplant Surg. 7 (6) (2007) 1661–1665.
[23] Y. Luo, B. Shi, Y. Qian, H. Bai, J. Chang, Sequential monitoring of TIM-3 gene

expression in peripheral blood for diagnostic and prognostic evaluation of acute rejection in renal graft recipients, Transplant. Proc. 43 (10) (2011) 3669–3674.
[24] K. Möller-Hackbarth, C. Dewitz, O. Schweigert, A. Trad, C. Garbers, S. Rose-John, J. Scheller, A disintegrin and metalloprotease (ADAM) 10 and ADAM17 are major sheddases of T cell immunoglobulin and mucin domain 3 (Tim-3), J. Biol. Chem. 288 (48) (2013) 34529–34544.
[25] X. Qiao, K. Jiang, J. Nie, K. Fan, Z. Zheng, J. Wang, J. Li, Increased expression of Tim-3 and its ligand Galectin-9 in rat allografts during acute rejection episodes, Biochem. Biophys. Res. Commun. 445 (3) (2014) 542–548.
[26] E.L. Naka, V.C. Ponciano, M.A. Cenedeze, A. Pacheco-Silva, N.O. Camara, Detection of the Tim-3 ligand, galectin-9, inside the allograft during a rejection episode, Int. Immunopharmacol. 9 (6) (2009) 658–662.
[27] Y.M. Li, Y.Y. Shi, Y. Li, L. Yan, J.T. Tang, Y.J. Bai, X.J. Wu, B. Dai, Y.G. Zou, L.L. Wang, Soluble Tim-3 and Gal-9 are associated with renal allograft dysfunction in kidney transplant recipients: a cross-sectional study, Int. Immunopharmacol. 55 (2018) 330–335.
[28] J.H. Lai, S.F. Luo, M.Y. Wang, L.J. Ho, Translational implication of galectin-9 in the pathogenesis and treatment of viral infection, Int. J. Mol. Sci. 18 (10) (2017).
[29] S. Merani, W. Chen, S. Elahi, The bitter side of sweet: the role of Galectin-9 in immunopathogenesis of viral infections, Rev. Med. Virol. 25 (3) (2015) 175–186.
[30] K.L. Clayton, M.B. Douglas-Vail, A.K.M.N. Rahman, K.E. Medcalf, I.Y. Xie, G.M. Chew, R. Tandon, M.C. Lanteri, P.J. Norris, S.G. Deeks, et al., Soluble T cell immunoglobulin mucin domain 3 is shed from CD8 + T cells by the sheddase ADAM10, is increased in plasma during untreated HIV infection, and correlates with HIV disease progression, J. Virol. 89 (7) (2015) 3723–3736.
[31] A. Kasamatsu, K. Uzawa, K. Shimada, M. Shiiba, Y. Otsuka, N. Seki, Y. Abiko, H. Tanzawa, Elevation of galectin-9 as an inflammatory response in the periodontal ligament cells exposed to Porphylomonas gingivalis lipopolysaccharide in vitro and in vivo, Int. J. Biochem. Cell Biol. 37 (2) (2005) 397–408.
[32] S.S.S. Heidt, A.S. Muthusamy, D. San Segundo, K.J. Wood, Pretransplant serum CXCL9 and CXCL10 levels fail to predict acute rejection in kidney transplant recipients receiving induction therapy, Transplantation 91 (8) (2011) e59–e61.
[33] M. Rotondi, A. Rosati, A. Buonamano, L. Lasagni, E. Lazzeri, F. Pradella, V. Fossombroni, C. Cirami, F. Liotta, G. La Villa, et al., High pretransplant serum levels of CXCL10/IP-10 are related to increased risk of renal allograft failure, Am. J. Transplant. 4 (9) (2004) 1466–1474.
[34] F.W. Vondran, K. Timrott, S. Kollrich, A.K. Steinhoff, A. Kaltenborn, H. Schrem, J. Klempnauer, F. Lehner, R. Schwinzer, Pre-transplant immune state defined by serum markers and alloreactivity predicts acute rejection after living donor kidney transplantation, Clin. Transpl. 28 (9) (2014) 968–979.
[35] M. Fernandez-Ruiz, P. Parra, T. Ruiz-Merlo, F. Lopez-Medrano, R. San Juan, N. Polanco, E. Gonzalez, A. Andres, J.M. Aguado, Association between baseline serum hepcidin levels and infection in kidney transplant recipients: potential role for iron overload, Transpl. Infect. Dis. 20 (1) (2018).
[36] M.G. Betjes, Immune cell dysfunction and inflammation in end-stage renal disease, Nat. Rev. Nephrol. 9 (5) (2013) 255–265.
[37] S. Kato, M. Chmielewski, H. Honda, R. Pecoits-Filho, S. Matsuo, Y. Yuzawa, A. Tranaeus, P. Stenvinkel, B. Lindholm, Aspects of immune dysfunction in end-stage renal disease, Clin. J. Am. Soc. Nephrol. 3 (5) (2008) 1526–1533.
[38] M.J. Sarnak, B.L. Jaber, Mortality caused by sepsis in patients with end-stage renal disease compared with the general population, Kidney Int. 58 (4) (2000) 1758–1764.
[39] P. Jayaraman, I. Sada-Ovalle, S. Beladi, A.C. Anderson, V. Dardalhon, C. Hotta, V.K. Kuchroo, S.M. Behar, Tim3 binding to galectin-9 stimulates antimicrobial immunity, J. Exp. Med. 207 (11) (2010) 2343–2354.
[40] F. Ren, J. Li, X. Jiang, K. Xiao, D. Zhang, Z. Zhao, J. Ai, C. Hou, Y. Jia, G. Han, et al., Plasma soluble Tim-3 emerges as an inhibitor in sepsis: sepsis contrary to membrane Tim-3 on monocytes, Tissue Antigens 86 (5) (2015) 325–332.