Clinical value of the sTim-3 level in chronic kidney disease

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Abstract. Chronic kidney disease (CKD) is a global disease that is harder to treat at a later stage. Therefore, early diagnosis and monitoring of CKD are crucial. T cell immunoglobulin and mucin domain molecule 3 (Tim-3) is a negative regulator of the T cell responses and it is involved in the immunomodulation of kidney disease. To date, only a small number of reports regarding serum soluble Tim-3 (sTim-3) in CKD are available. In the present study, the serum levels of sTim-3 in patients with CKD at different stages and the levels of sTim-3 in the early diagnosis and monitoring of CKD were analyzed. A highly sensitive time-resolved fluorescence immunoassay was performed to quantify sTim-3 levels in 318 patients with CKD and 114 healthy individuals. The serum levels of sTim-3 in patients with CKD (33.47±20.77 ng/ml) were significantly higher than those in the healthy individuals group (8.32±3.23 ng/ml; P<0.0001). As CKD progressed from stage G1 to G5, the serum sTim-3 level gradually increased (P<0.0001). A cut-off value of 13.63 ng/ml for the sTim-3 concentration was effective in diagnosing patients with CKD (area under the receiver operating characteristic curve, 0.9176; sensitivity, 79.87%; specificity, 96.49%). At this critical value, the positive detection rate of CKD in the early stages (G1 + G2), G3, G4 and G5 was 55.70, 77.78, 84.44 and 92.86%, respectively. In conclusion, the serum sTim-3 levels in patients with CKD were significantly higher than those in the healthy individuals group. As CKD progressed from G1 to G5, the serum sTim-3 concentration gradually increased, facilitating the monitoring of the progression of CKD. In addition, serum sTim-3 had an auxiliary effect that was useful in the early diagnosis of CKD. The positive detection rate of CKD in the early stages was 55.70%, which can assist other clinically common kidney disease indicators.

Introduction

Chronic kidney disease (CKD) is a worldwide disease that affects >10% of the world's population (1). CKD can be caused by several clinical conditions, including renal tubular damage, renal vascular disease, and primary and secondary glomerulonephritis. The progression of CKD can be divided into five stages according to the glomerular filtration rate (GFR) (2). Once CKD progresses to stage 5, it is hard to treat and requires timely initiation of renal replacement therapy (3). Early detection and intervention can markedly reduce CKD-related clinical complications, which may be reflected by improvement of the survival rate. Currently, tissue biopsy is the gold standard in the clinical evaluation of CKD (4). However, the invasiveness of this method can cause discomfort and possibly lead to complications (5). Serum creatinine (CREA) and proteinuria are common serum biomarkers (6) of kidney disease; however, their sensitivity and specificity are limited. In CKD and acute kidney injury, the sensitivity of serum creatinine is only 17% (7) and they have little effect on early detection or monitor disease progression (8). Therefore, more effective serum markers for the early diagnosis and progression monitoring of CKD are required.

The immunomodulatory role of T cell immunoglobulin and mucin domain molecule 3 (Tim-3) in kidney disease has attracted considerable interest. Tim-3 is a member of the TIM family of immunomodulatory proteins and was first found to be

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Abbreviations: AKI, acute kidney injury; CKD, chronic kidney disease; GFR, glomerular filtration rate

Key words: CKD, soluble T cell immunoglobulin and mucin domain molecule 3, time-resolved fluorescence immunoassay, diagnosis, biomarker

expressed on T helper (Th) 1 cells (9). Mast cells, natural killer cells, dendritic cells, Th17 cells and cytotoxic $CD8^+$ T cells can express Tim-3 (10-14). Tim-3 can be found in two forms: A membrane-bound Tim-3 and a soluble Tim-3 (sTim-3) form (15). Membrane-bound Tim-3 can be cleaved from the cell surface by a disintegrin and metalloproteinase domain-containing protein 10 and 17 to generate sTim-3 (16). It has been reported that Tim-3 expression is increased in the macrophages of diabetic mice, and this increase is positively associated with the severity of renal dysfunction (17). In immune-related nephropathy, Tim-3 is highly expressed in the renal tissues of patients with IgA nephropathy and in the $CD14^+$ monocytes present in the peripheral blood of patients with systemic lupus erythematosus nephropathy (18,19). In nephrotoxic serum nephritis, Tim-3 negatively regulates the activation of renal macrophages. Furthermore, blocking Tim-3 increases the number of infiltrating inflammatory cells in the kidneys, thereby aggravating nephritis. This finding suggests that Tim-3 exerts a protective role in the process of nephritis (20). To the best of our knowledge, studies on the involvement of Tim-3 in kidney diseases have mainly focused on its membrane-bound form (21-23). In the present study, a highly sensitive time-resolved fluorescence immunoassay was used to detect serum sTim-3 levels in patients with CKD. The serum levels of sTim-3 in patients with CKD at different stages and the role of sTim-3 in the early diagnosis and monitoring of CKD were analyzed.

Materials and methods

Reagents and instruments. Two monoclonal antibodies against different epitopes of Tim3, capture (Cat.No:SEK10390-R024; rabbit McAb) and detection antibody (Cat.No:SEK 10390-MM04; mouse MAb), and TIM-3 protein, Human, recombinant (Cat. No:SEK 10390-H08H, His Tag); were purchased from Sino Biological Inc. ProClin 300 (48915-U), Tris-HCl (1082191), Sephadex-G50 (G5080), NaCl (S9888), Na_2CO_3 (S7795), $NaHCO_3$ (S6014), diethylenetriaminepentaacetic acid (DTPA; 791274P), 2-naphthoyltrifluoroacetone (β-NTA; 343633), Triton X-100 (X100P3) and Tween-20 (P9416) were purchased from Sigma-Aldrich; Merck KGaA. BSA (240GR100) was purchased from Guangzhou Saiguo Biotech Co., Ltd.

A time-resolved immunofluorescence analyzer was purchased from Guangzhou Daan Gene Co., Ltd. and 96-well plates were purchased from Xiamen Yunpeng Technology Development Co., Ltd.

Buffer composition. Coating buffer (50 mmol/l Na_2CO_3-$NaHCO_3$; pH 9.6); elution buffer (50 mmol/l Tris-HCl, 0.2% BSA and 0.05% ProClin 300; pH 7.8); washing buffer (50 mmol/l Tris-HCl, 0.9% NaCl, 0.02% Tween-20 and 0.01% ProClin 300; pH 7.8); blocking solution (50 mmol/l Tris-HCl, 0.9% NaCl, 1% BSA and 0.05% ProClin 300; pH 7.8); labeling buffer (50 mmol/l Na_2CO_3-$NaHCO_3$; pH 9.0); analysis buffer (50 mmol/l Tris-HCl, 0.9% NaCl, 0.5% BSA, 0.0008% DTPA, 0.0005% Phloxine B, 0.01% Tween-20 and 0.05% ProClin 300; pH 7.8); and enhancement solution (15 μmol/l β-NTA and 50 μmol Triton X-100; pH 3.2).

Serum samples. Serum samples were collected from 318 patients with CKD between June 2020 and December 2021 either at Wuxi People's Hospital (Wuxi, China) or at Zhejiang Provincial People's Hospital (Hangzhou, China). The inclusion criteria were: i) Age >18 years; and ii) presence of CKD, which was defined as estimated GFR (eGFR) <90 ml/min/1.73 m^2. The exclusion criteria were: i) Kidney transplantation; and ii) renal dialysis with acute kidney injury (AKI).

According to the CKD staging standard proposed by the Kidney Disease Prognosis Quality Initiative Working Group of the American Kidney Disease Foundation in 1999 (24), the patients were divided into five groups according to their eGFR (G1, eGFR ≥90 ml/min/1.73 m^2; G2, 89 ml/min/1.73 m^2 ≥eGFR ≥60 ml/min/1.73 m^2; G3, 59 ml/min/1.73 m^2 ≥eGFR ≥30 ml/min/1.73 m^2; G4, 30 ml/min/1.73 m^2 ≥eGFR ≥15 ml/min/1.73 m^2; and G5, eGFR <15 ml/min/1.73 m^2). The number of samples from different stages was random. In addition, serum samples were collected from 114 healthy individuals between June 2020 and December 2021 at Wuxi People's Hospital (Wuxi, China). Demographic characteristics are listed in Table I. The inclusion criteria for the control group were: i) Hospital admission for general physical examination; ii) no history of kidney disease; and iii) eGFR >90 ml/min/1.73 m^2. The exclusion criteria for the control group were as follows: i) History of kidney disease; ii) eGFR ≤90 ml/min/1.73 m^2; and iii) have kidney disease or other disease.

Venous blood (5 ml) was collected from each participant and centrifuged at 1,000 g for 10 min at 2-6˚C. The supernatant (serum) was stored at -80˚C.

Blood test results for CREA, eGFR, albumin (ALB), uric acid and urea levels were provided by the hospital. CREA, ALB, URIC and Urea were determined by biochemical analyzer (Beckman coulter AU. eGFR is obtained as follows: Male eGFR=(140-age)xweight(kg)x1.23/CREA (umol/l); female eGFR=(140-age)xweight(kg)x1.03/CREA (umol/l).

The study was approved by the Ethics Committee of the Wuxi People's Hospital Affiliated to Nanjing Medical University (NMU2018211, Wuxi, China) and Ethics Committee of the Zhejiang Provincial People's Hospital (2018KT063, Zhejiang, China). Written informed consent was obtained from all registered participants.

Time-resolved fluorescence immunoassay double antibody sandwich method for serum sTim-3 detection. The experimental protocol has been described previously (25). Briefly, the steps were as follows: *Antibody coating*. The capture antibody was diluted to 2 μg/ml with a coating buffer (dilution, 1:500), and 100 μl of the diluted capture antibody solution was added to each well of the 96-well plate. The solutions were incubated overnight at 4˚C. The plate was washed once with washing buffer, and 150 μl blocking solution was added to each well. After blocking at room temperature for 2 h, the blocking solution was discarded. After drying, the antibody-coated plate was stored at -20˚C until use.

Labeling antibody. The detection antibody (300 μg) was added to an ultrafiltration tube. Through ultrafiltration, the buffer of the antibody to be detected was converted into a labeling buffer with pH 9.0. The collected antibody was mixed with 30 μl 2 mg/ml diethylenetriaminetriacetic acid-Eu^{3+}, and the mixture was incubated at 30˚C overnight. The next day, the labeled antibody was purified with Sephadex G50 and elution

Table I. Comparison of various parameters in the different GFR stages of chronic kidney disease.

Index	Control	G1	G2	G3	G4	G5	P-value
Sex, n (M/F)	55/59	20/24	17/18	24/30	23/22	69/71	0.2700
Age, years	53.12±15.02	45.31±14.14	53.16±15.76	65.98±17.32	64.1±17.07	59.89±17.16	0.0020
eGFR, ml/min/ 1.73 m^2	120.80±13.56	104.60±10.81	76.93±8.18	41.56±6.95	20.57±4.51	7.26±3.11	<0.0001
Urea, mmol/l	4.90±1.55	9.45±9.94	9.93±14.74	9.66±4.09	15.88±8.31	22.24±9.16	<0.0001
CREA, μmol/l	85.81±15.12	164.94±261.61	88.53±21.27	143.61±45.46	229.30±81.28	655.39±290.41	<0.0001
ALB, g/l	44.89±7.32	35.78±8.36	41.66±33.84	31.63±7.64	31.43±6.67	7.26±3.11	0.0940
URIC, μmol/l	363.83±40.66	391.70±129.29	356.50±126.77	332.03±132.12	382.65±155.62	366.91±122.98	0.8030
Urea/CREA	0.06±0.02	0.08±0.03	0.08±0.04	0.07±0.03	0.10±0.19	0.04±0.02	<0.0001
sTim-3, ng/ml	8.32±3.23	20.28±19.99	24.74±20.43	29.10±17.33	35.06±20.47	40.97±17.80	<0.0001

Age, eGFR, Urea, CREA, ALB, URIC, Urea and sTim-3 are presented as the mean ± SD. The sTim-3 concentration and various clinical parameters in different GFR stages were analyzed using the Jonckheere-Terpstra test. P<0.05 was considered to indicate a statistically significant difference. ALB, albumin; CREA, serum creatinine; eGFR, estimated glomerular filtration rate; F, female; M, male; sTim-3, soluble T cell immunoglobulin and mucin domain molecule 3; URIC, uric acid.

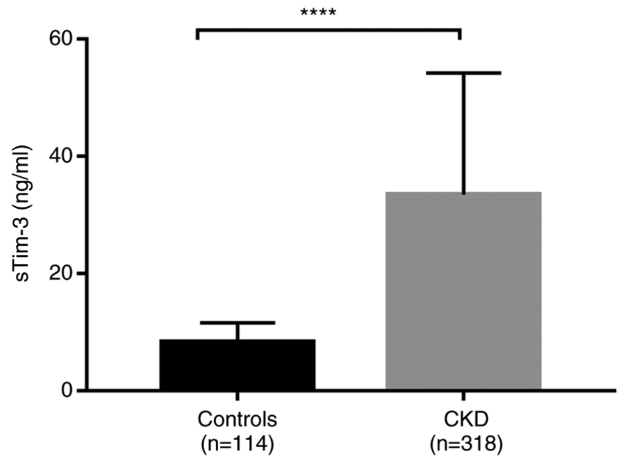

Figure 1. Concentration of sTim-3 in serum of healthy controls and patients with CKD. ****P<0.0001. sTim-3, soluble T cell immunoglobulin and mucin domain molecule 3; CKD, chronic kidney disease. Unpaired Student's t-test was performed to compare the levels of serum indicators in patients and controls.

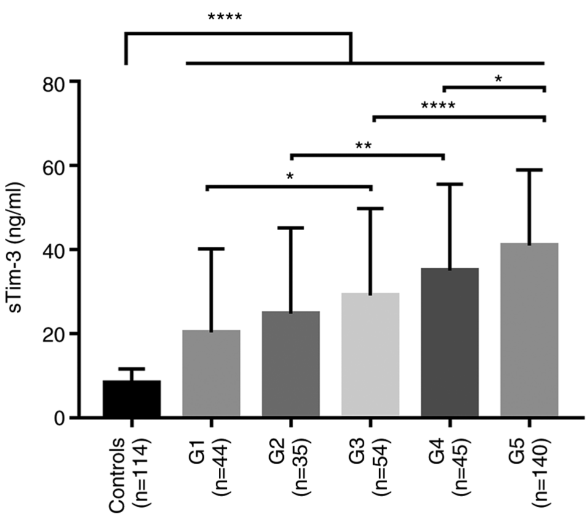

Figure 2. sTim-3 concentration in different glomerular filtration rate stages of patients with chronic kidney disease. P<0.05 was considered to indicate a statistically significant difference. *P<0.05, **P<0.01, ****P<0.0001. sTim-3, soluble T cell immunoglobulin and mucin domain molecule 3.

buffer. Finally, the Eu^{3+}-McAb-labeled antibody was collected and stored at -20˚C.

sTim-3 antigen dilution. sTim-3 antigen was diluted with an analysis buffer to different concentrations (6.25, 12.5, 25, 50 and 100 ng/ml).

Determination of sTim-3 concentration in serum. The standard solution or serum sample (100 μl) was added into a 96-well microtiter plate coated with the anti-Tim-3 capture antibody. The plate was incubated at 37˚C for 1 h with shaking and washed twice with washing buffer (room temperature and 5 sec each). Subsequently, 100 μl Eu^{3+}-McAb (diluted 1:1,000 with analysis buffer) was added to each well, incubated at 37˚C for 1 h, and then washed six times with washing buffer. Furthermore, ~100 μl enhancement solution was added to each well, and the plate was incubated with shaking for 3 min at 37˚C. Finally, fluorescence was analyzed using the time-resolved immunofluorescence analyzer (Guangzhou Daan Gene Co., Ltd., Guangzhou, China).

Statistical analysis. Data are presented as the mean ± standard deviation or quartile differences. Statistical analysis was performed using SPSS software version 21.0 (IBM Corp.). Unpaired Student's t-test was performed to compare the levels of serum indicators in patients and controls. One-way ANOVA and a post hoc test (Tamhane's T2) for multiple comparisons were used to analyze the differences among groups. sTim-3 levels and various clinical parameters in CKD stages defined by eGFR interval were compared using the Jonckheere-Terpstra test. The correlations among the values were determined by calculating Spearman's correlation coefficient. GraphPad Prism 7.0 (GraphPad Software, Inc.) was used to draw the

Table II. Multivariate regression analysis of sTim3 and other clinical indicators in patients with chronic kidney disease.

Clinical indicator	P-value	OR	95% CI
Sex	0.124	0.313	0.071-1.374
Age	0.009	0.930	0.881-0.982
eGFR	<0.001	0.866	0.806-0.931
Urea	0.286	1.428	0.742-2.749
CREA	0.990	1.000	0.988-1.012
ALB	0.014	0.866	0.772-0.971
URIC	0.496	1.002	0.995-1.010
sTim3	0.030	1.144	1.013-1.292

OR, odds ratio; eGFR, estimated glomerular filtration rate; CREA, serum creatinine; ALB, albumin; URIC, uric acid; sTim3, soluble T cell immunoglobulin and mucin domain molecule 3.

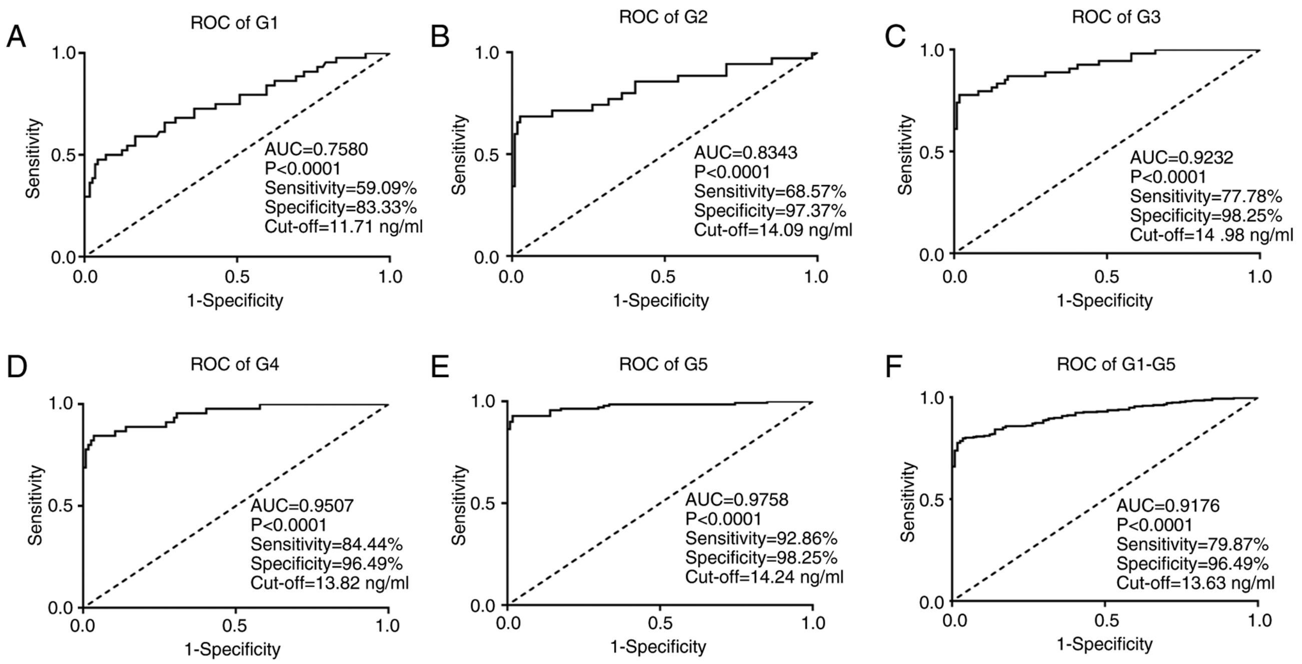

Figure 3. ROC analysis of serum sTim-3 suggests that this has diagnostic value for healthy controls and patients with CKD. (A) ROC curve of serum sTim-3 in healthy controls and patients with CKD (G1). (B) ROC curve of serum sTim-3 in healthy controls and patients with CKD (G2). (C) ROC curve of serum sTim-3 in healthy controls and patients with CKD (G3). (D) ROC curve of serum sTim-3 in healthy controls and patients with CKD (G4). (E) ROC curve of serum sTim-3 in healthy controls and patients with CKD (G5). (F) ROC curve of serum sTim-3 in healthy controls and patients with CKD (G1-G5). ROC, receiver operating characteristic; sTim-3, soluble T cell immunoglobulin and mucin domain molecule 3; CKD, chronic kidney disease; AUC, area under the curve.

receiver operating characteristic curve for the determination of the best cut-off value of serum sTim-3, as well as for the evaluation of the performance of sTim-3 in differentiating patients with CKD from the healthy control group. P<0.05 was considered to indicate a statistically significant difference.

Results

Differences between the serum sTim-3 levels of healthy controls and patients with CKD. As shown in Fig. 1, the serum sTim-3 concentrations of patients with CKD (33.47±20.77 ng/ml) were significantly higher than those of healthy controls (8.32±3.23 ng/ml; P<0.0001).

Relationship between sTim-3 levels and other clinical indicators in the serum and the stages of CKD. To explore the relationship between serum sTim-3 and the progression of CKD, patients with CKD were divided into five groups according to their disease stage (G1-G5). The Jonckheere-Terpstra test was used to compare sTim-3 levels and changes in various clinical parameters at different GFR stages. The results are shown in Table I. As the disease stage progressed, sTim-3 (P<0.0001), urea (P<0.0001) and CREA (P<0.0001) showed upward trends, whereas urea/CREA (P<0.0001) showed a downward trend. As shown in Fig. 2, the serum sTim-3 concentration gradually increased from the healthy control group to the G5 stage. sTim-3 concentrations of patients with G3 stage

Table III. Positive detection rate of different stages of chronic kidney disease progression.

Cut-off value, ng/ml	Control, n (%) (n=114)	G1 and G2, n (%) (n=79)	G3, n (%) (n=54)	G4, n (%) (n=45)	G5, n (%) (n=140)	Total, n (%) (n=318)
13.63	4 (3.51)	44 (55.70)	42 (77.78)	38 (84.44)	130 (92.86)	254 (79.87)

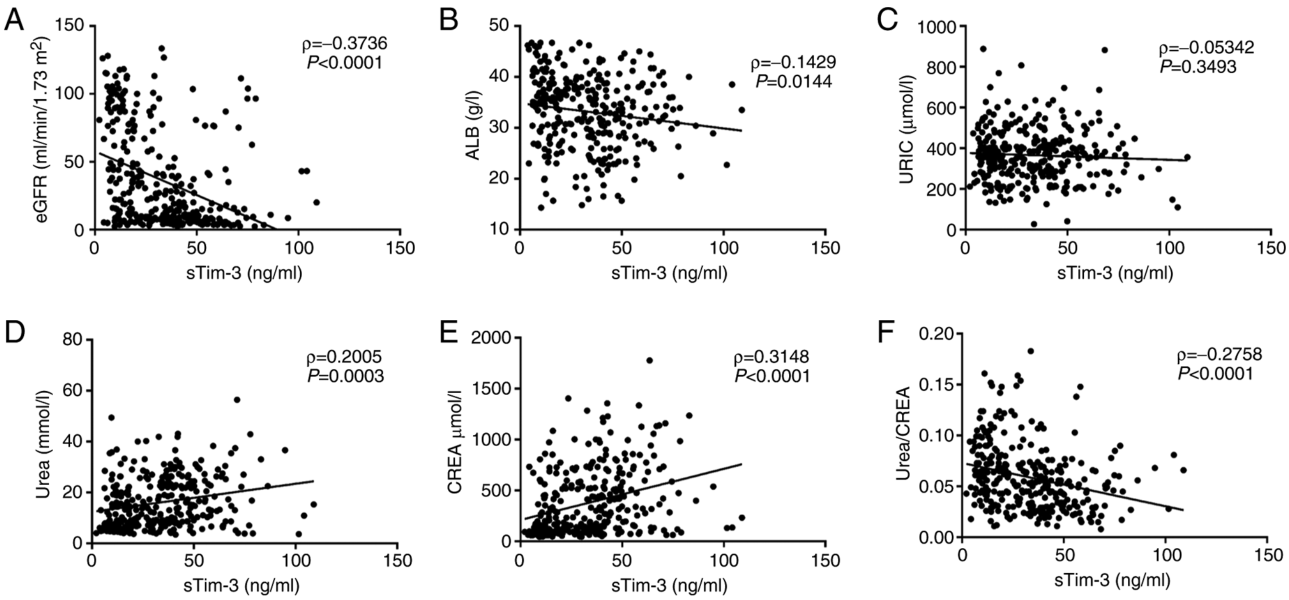

Figure 4. Relationship between serum sTim-3 levels and chronic kidney disease indexes in patients. (A) Correlation analysis of serum levels of sTim-3 and eGFR revealed a significant negative correlation. (B) Correlation analysis of serum levels of sTim-3 and ALB indicated a significant negative correlation. (C) Correlation analysis of serum levels of sTim-3 and URIC revealed no significant correlation. (D) Correlation analysis of serum levels of sTim-3 and urea indicated a significant positive correlation. (E) Correlation analysis of serum levels of sTim-3 and CREA revealed a significant positive correlation. (F) Correlation analysis of serum levels of sTim-3 and urea/CREA indicated a significant negative correlation. sTim-3, soluble T cell immunoglobulin and mucin domain molecule 3; eGFR, estimated glomerular filtration rate; CREA, serum creatinine; ALB, albumin; URIC, uric acid.

were significantly higher than those of G1 (P<0.05); sTim-3 concentrations of patients with G4 stage were significantly higher than those of G2 (P<0.001); sTim-3 concentrations of patients with G5 stage were significantly higher than those of G3 (P<0.0001); sTim-3 concentrations of patients with G5 stage were significantly higher than those of G4 (P<0.05).

Multivariate regression analysis of the sTim3 levels and other clinical indicators for patients with CKD. The multivariate regression analysis performed on the sTim-3 levels in patients with CKD (total) and other clinical indicators (shown in Table II) suggested that eGFR [odds ratio (OR), 0.866; 95% CI, 0.806-0.931], ALB (OR, 0.866; 95% CI, 0.772-0.971), age (OR, 0.930; 95% CI, 0.881-0.982) and sTim-3 (OR, 1.144; 95% CI, 1.013-1.292) could be independent factors (P<0.05).

Levels of serum sTim-3 in the early diagnosis of CKD. A receiver operating characteristic curve was drawn and used to evaluate the diagnostic value of serum sTim-3 in patients with CKD, especially its value in terms of early diagnosis. The results are shown in Fig. 3. From G1 to G5, the area under the curve gradually increased (0.7580, 0.8343, 0.9232, 0.9507 and 0.9758, respectively, from G1 to G5). As CKD developed, the diagnostic value of sTim-3 increased. This further suggests that the concentration of serum sTim-3 is closely related to the development of CKD.

In the diagnosis of CKD, the G1-G5 stages were diagnosed (Table III) with the use of serum sTim-3 levels. According to the Youden index shown in Fig. 3F, the cut-off value was 13.63 ng/ml. The positive detection rate of early CKD (G1 and G2) was 55.70%. The positive rate in the normal control group was 3.51%.

Correlation between serum sTim-3 levels and other biochemical indicators. A correlation diagram was generated to explore the correlation between serum sTim-3 and the other clinical indicators of CKD. As shown in Fig. 4, the serum sTim-3 concentrations of the patients with CKD were significantly positively correlated with urea (ϱ=0.2005; P=0.0003) and CREA (ϱ=0.3148; P<0.0001). Significant negative correlations with eGFR (ϱ=-0.3736; P<0.0001), ALB (ϱ=-0.1429; P=0.0144) and urea/CREA (ϱ=-0.2758; P<0.0001) were observed. There is no significant correlation between serum sTim-3 and URIC (ϱ=-0.0534; P=0.3493).

Discussion

CKD is defined as 'abnormal structure or function of the kidney, lasting >3 months, with health effects' (26). Given that the early symptoms of CKD are not obvious, its prevention and early diagnosis remain challenging. Currently, the GFR is the best indicator for evaluating renal function. The urine isotope collection method is the 'gold standard' for measuring GFR. However, this method is time-consuming, expensive and is usually estimated using the formula for an endogenous filtration marker (CREA) (27); therefore, this method has limited value in the early diagnosis of CKD. Therefore, the present study explored novel serum markers, aiming to improve the early diagnosis of CKD.

Tim-3 is an immune checkpoint molecule (9), serving an immunomodulatory role in a variety of diseases, such as viral infections (28,29), systemic lupus erythematosus (30) and tumors (31). However, the clinical value of serum sTim-3 in CKD remains unclear. In the present study, the serum levels of sTim-3 in patients with CKD were quantified and the clinical value of sTim-3 in CKD was explored. The results of the present study demonstrated that sTim-3 levels in the sera of patients with CKD were significantly higher than those in the sera of healthy people. The serum sTim-3 levels increased with the progression of CKD, suggesting that serum sTim-3 could be used to monitor the progression of CKD.

Inflammation serves an important role in the progression of CKD, and circulating monocytes and endothelial cells are the main sources of inflammatory cytokines (32). Tim-3 can negatively regulate the production of anti-inflammatory factors such as IL-10 and proinflammatory factors (TNF-α and IL-6) (33). Therefore, Tim-3 could be involved in CKD by regulating inflammation.

AKI can also lead to the development of CKD (34). An early feature of AKI is that immune cells accumulate in the kidney, and immune cells cause further damage through inflammatory mechanisms (35). The downregulation of Tim-3 impairs the function of regulatory T (Treg) cells in AKI (36). Therefore, Tim-3 may also affect AKI by regulating Treg cells and further participate in the progression of CKD. In addition, the imbalance between Th1 and Th2 could have a central role in the pathogenesis of immune-related nephropathy (30). The combination of Tim-3 and its ligand galectin 9 promotes Treg activation and increases the expression level of Tim-3 proteins during activation. The increase in Tim-3 levels can induce Th1 cell apoptosis, inhibit Th1-type immune responses (37) and promote the shift of the Th1/Th2 balance towards Th2 (38), thereby participating in the development of nephropathy. The level of serum sTim-3 may reflect the level of membrane-bound Tim-3 (12). Therefore, the aforementioned factors may explain why serum sTim-3 levels gradually increased with CKD stage.

Currently, eGFR is the best indicator for measuring renal function (8), while CREA is the most commonly used biomarker of renal function, as it can be used to estimate GFRs (8). Given that the daily production and excretion of CREA are relatively constant, an increase in the CREA level indicates a decrease in the GFR (39). In general, the severity of CKDincreases with CREA. Due to continuous damage to the kidneys, the function of excreting wastes is reduced, which causes the accumulation of toxins, including CREA, in the body, resulting in an increase in the urea levels (40). The present study demonstrated that the level of serum sTim-3 in patients with CKD was significantly correlated with eGFR, CREA and urea indicators, suggesting that serum sTim-3 could be used to evaluate the degree of kidney damage and CKD progression. In addition, serum sTim-3 can be effective in the diagnosis of CKD and showed a sensitivity of 79.87% and specificity of 96.49%. The cut-off value of serum sTim-3 was 13.63 ng/ml, and the positive diagnosis rate for CKD in the G1 and G2 stages was 55.70%. The diagnostic eGFR criteria for the G1 stage of CKD and for healthy controls are both ≥90 ml/min/1.73 m^2, making it difficult to diagnose the early stage of CKD. The present study suggests that serum sTim-3 can be employed in the early diagnosis of CKD.

In summary, the present study revealed that the serum sTim-3 level was a useful clinical reference value for the diagnosis of CKD, especially in the early diagnosis of the disease and in the evaluation of its development, thus providing novel insights into the role of serum sTim-3 in kidney diseases. The present study demonstrated that serum sTim-3 levels were closely related to renal function indicators (eGFR and CREA). The concentrations of sTim-3 in the sera of patients with CKD were higher than those in the sera of healthy individuals. As the stage of CKD increased as the course of the disease worsened, sTim-3 concentrations gradually increased, thereby allowing monitoring of CKD progression. In addition, the quantitative determination of serum sTim-3 may improve the early diagnosis of CKD.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LC, YQ, BH, XY, BL and SZ wrote the manuscript and analyzed data. BH, XZ, YW, JJ, LW and XL designed and performed the experiments. BH, JJ and LW confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). This manuscript is part of a study that was approved by the Institutional Research Ethics Committee of the Wuxi People's Hospital Affiliated to Nanjing Medical University (approval no. NMU2018211; Wuxi, China) and the Zhejiang Provincial People's Hospital (approval no. 2018KT063; Hangzhou, China), and written informed consent was obtained from all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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