

Characteristics of T lymphocyte subsets and their apparent effect on infection in patients with chronic kidney disease

Yali Zhang

Renal Division, Inner Mongolia People's Hospital, Hohhot, Inner Mongolia 010017.

Ying Ji

Renal Division, Inner Mongolia People's Hospital, Hohhot, Inner Mongolia 010017.

Lei Yu (✉ lanlife@163.com)

Renal Division, Inner Mongolia People's Hospital, Hohhot, Inner Mongolia 010017.

Ye Zhao

Renal Division, Inner Mongolia People's Hospital, Hohhot, Inner Mongolia 010017.

Guoping Liu

Renal Division, Inner Mongolia People's Hospital, Hohhot, Inner Mongolia 010017.

Lina Dong

Renal Division, Inner Mongolia People's Hospital, Hohhot, Inner Mongolia 010017.

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Abstract

Background

Chronic kidney disease (CKD) is associated with Immune deficiency. T lymphocytes are significant components of the adaptive immune system. Previous studies have found dysfunction of T lymphocytes in patients with CKD, but the changing trend of the absolute number of T lymphocytes and the relationship with infection has been still unclear in CKD.

Methods

We collected 447 patients with CKD, tested the absolute counts of T lymphocyte subsets, explored the relationship between the absolute count of T lymphocytes and renal function, and explored these impacts on infection.

Results

absolute counts of T lymphocyte gradually decrease as CKD progresses. The patients with infection demonstrated fewer T lymphocyte cells compared with non-infected patients. $CD4^+$ (cluster of differentiation 4) T lymphocytes are closely related to infection and $CD4^+ T < 469$ cells/ μ l has a certain value in predicting infection in patients with CKD.

Conclusion

With the gradual decline of renal function in CKD patients, the level of T lymphocytes gradually decreased and the risk of infection gradually increased. The $CD4^+T$ absolute count has a specific predictive power for infection in patients with chronic kidney disease. Our results suggest a new feasible strategy for preventing infections in CKD patients.

Background

Chronic kidney disease (CKD) is defined by abnormalities in kidney structure and/or function for more than three months¹. CKD is divided into five stages according to the glomerular filtration rate (GFR). A general population-based survey for CKD was performed in China, and the prevalence of CKD was 10.8%². For a progressive disease, CKD has received increased attention as a leading public health problem in worldwide. A variety of risk factors could cause the deterioration of renal function. Infection is the most common and dangerous complication in the CKD patients. The incidence of infection in early stages of CKD patients is three to five times than non-CKD patients³. In the United States, older than 65 years CKD individuals hospitalized for infection are 8 to 20 times frequency and longer stay in hospital than general

population⁴. Furthermore, infection is a more serious complication and an important risk factor for death. In maintenance dialysis patients, infections constitute the second leading cause of mortality⁵, accounting for 8% of deaths.

T lymphocytes play a key role in fighting infection by activating immunoreaction and stimulating the adaptive immune response. As an important indicator for level of cellular immune, The analysis of T-lymphocyte subsets has received extensive attention for the diagnosis and treatment in immune diseases^{6,7}. But, in present, most studies in this field only forced on acquired immunodeficiency syndrome (AIDS) . It has been suggested CD4⁺ (cluster of differentiation 4⁺) T lymphocyte counts could assess immunity, such as CD4⁺ T count below 200 would be a great predictor for the development of HIV-related infection.

Although it is known that CKD patients may have abnormal cellular immune function, most studies only targeted lymphocyte abnormalities in patients with uremia or end-stage renal disease (ESRD). The studies about the T lymphocytes in CKD patients are still rare^{8,9}. Furthermore, almost no study explored the absolute count of T cells to assess immune function and predict the risk of infection in CKD patients. Therefore, the present study focused on CKD patients and analyzed lymphocyte count characteristics, in order to find a new feasible strategy for preventing infections in CKD patients.

Methods

Patients

A total of 447 CKD patients were recruited in our renal division. All patients provided written informed consent. The Medical Ethics Committee of Inner Mongolia People's Hospital approved the study, which was conducted according to the declaration of Helsinki. All methods were executed in accordance with the relevant guidelines and regulations.

Inclusion and exclusion criteria

Four inclusion criteria were used for this study: **I)** Meet the diagnostic criterions of K/DOQI (The National Kidney Foundation's Kidney Disease Outcomes Quality Initiative) guidelines for CKD; **II)** The patient's information is complete, including past history, current medical history, laboratory tests, medication records, and clinical diagnosis. **III)** The consciousness of patients is clear, and correct self-information can be expressed; **IV)** Patients gave informed consent to this study.

Five exclusion criteria were employed: **I)** Patients with shock, severe heart failure or arrhythmia were excluded; **II)** Patients with severe malignant tumors, advanced cancers and other severely hypercatabolic, cachexia, and endangered stage were excluded; **III)** Exclude patients with a history of stem cell or organ transplantation; **IV)** Exclude patients with tonsillectomy; **V)** Patients diagnosed with AIDS were excluded.

Flow cytometry analyses

The BD (Becton,Dickinson and Company) FACSCalibur system can quickly perform routine tasks including immunophenotyping, absolute counting, residual white blood cell enumeration, stem cell analysis and DNA analysis. The absolute numbers of lymphocytes were calculated as laboratory manual of BD FACSCalibur flow cytometer. All subjects took 1ml of venous blood into a heparin anticoagulant tube on an empty stomach. . Using reverse injection method, added 50ul venous blood to an absolute counting tube (Trucount Absolute Count Tube, containing a known number of fluorescent beads) .Added 20ul of CD3/CD8/CD45/ CD4 four-color fluorescent antibodies into the absolute counting tube, mixed by vortexing, and placed at room temperature in the dark for 15-20 minutes. Then, added 450ul of 1 × FACS hemolysin, mixed thoroughly, and placed at room temperature in the dark for 15 minutes. A total of 10,000 fluorescence events was counted by the BD FACSCalibur flow cytometer with Multiset software.

Grouping standard:

1. Chronic kidney disease was classified into 5 stages according to the K/DOQI guideline;
2. To exclude the effects of hormones and immunosuppressants, as screening case group, patients with autoimmune diseases (systemic lupus erythematosus, vasculitis, etc.) or treating with hormones or immunosuppressants within 2 years were excluded;
- The patients were divided into bacterial infected and non-infected groups. The criteria of the bacterial infection: a) fever; b) the peripheral white blood cell count increased or decreased, and the neutrophil ratio increased; c) procalcitonin (PCT) increased (normal value <0.1ng / ml); d) with or without bacteremia (pathogenic bacteria detected in blood culture) ;e) with clinical manifestations of respiratory infections or urinary tract infections or skin infections. At same time, all of the above criteria were met to diagnose a bacterial infection;
1. According to the low reference value for the absolute count of lymphocytes (CD4⁺ absolute count 410 cells/μl, CD3⁺ absolute count 690 cells/μl, CD8⁺ absolute count 190 cells/μl), the study cases and screening cases are divided into two groups.

Statistical Analysis

The continuous variables with normal distribution were presented as mean ± standard deviation. Normality of continuous variables was tested by the Kolmogorov-Smirnov and Shapiro Wilk tests. The quantitative variables were estimated by t-test (variables with normal distribution) or Mann-Whitney U test (variables with non-normal distribution). For categorical variables, Pearson's chi-squared (χ^2) test was performed. Based on the variables in univariate analysis, multivariate analysis with logistic regression was conducted to identify independent risk factors. Statistical analyses were performed with SPSS12.0 software (SPSS Inc., Chicago, Illinois, USA). Receiver Operating Characteristic (ROC) curves was used for predicting cutoff values. Scatter plots and histogram plots were prepared in GraphPad Prism 6 (GraphPad Software, Inc., USA). P < 0.05 was considered statistically significant.

Results

T-lymphocyte count gradually decreased with the decline of renal function

The absolute counts of CD3⁺, CD4⁺, and CD8⁺ T lymphocytes in all CKD patients and screening patients with different stages of CKD were analyzed. With the decline of renal function, the absolute counts of CD3⁺, CD4⁺, and CD8⁺ T lymphocytes gradually decreased (Fig 1).

T lymphocyte levels are lower in bacterial infected patients

Among 447 patients with chronic kidney disease, the absolute counts of CD3⁺, CD4⁺, and CD8⁺ T cells in the bacterial infected group were lower than non-infected group (CD3⁺ T cells: 1294.12 ± 756.82 vs 1532.66 ± 891.26 ; CD4⁺ T cells: 713.70 ± 493.78 vs 833.39 ± 567.94 ; CD8⁺ T cells: 542.45 ± 363.44 vs 645.04 ± 439.98 , $P < 0.05$, Fig2). The results showed that the decrease of T lymphocyte levels was associated with infection. Furthermore, In the 165 screening cases, the absolute count of CD4⁺ in the infected group was lower than the non-infected group (643.14 ± 359.24 vs 757.23 ± 398.98 , $P < 0.05$, Fig2). It is inferred that the absolute CD4⁺ T count was more sensitive indicator for predicting bacterial infection.

The infection rate was significantly higher in the CD4⁺ T<410 cells /μl group

In 477 patients with chronic kidney disease, the infection rate was higher in the CD4⁺ T<410 cells /μl group than in the CD4⁺ normal group ($P < 0.05$). the same result was found in screening cases. The infection rate was still significantly higher in the CD4⁺ T<410 cells /μl group than in the normal group ($P < 0.05$). It is suggested that CD4⁺ T<410 cells / μl in patients with CKD would significantly increase the risk of infection. Table 1

Multivariate logistic regression for infection in CKD patients

Multivariate logistic regression was performed in 447 patients with chronic kidney disease. Infection was dependent variable, and gender, age, eGFR, the absolute counts of CD3⁺, CD4⁺, and CD8⁺ T cells were independent variables. A stepwise logistic regression model (forward likelihood ratio) was used with the standard: $P\text{-value} \leq 0.05$, independent variable entered into the equation, and $P\text{-value} > 0.1$, independent variable removed. CD4⁺ T absolute count [OR 1.584, (95% CI 1.04-2.41)] and eGFR[OR 1.00 (95% CI 0.99-1.00)] were independent risk factors for infection (Table 2). Similarly, in the 165 screening cases, the above factors were analyzed by logistic regression. CD4⁺ T absolute count still significantly associated with infection [OR 3.47 (95% CI 1.55-7.77), Table 2]. Therefore, CD4⁺ T absolute count was independent risk factors for infection.

To better predict bacterial infection in CKD patients, the optimal cutoff value was determined in CD4⁺ absolute count

The ROC curves of CD4⁺ T could reveal a certain predictive ability for infection in patients with chronic kidney disease. The AUC area (area under the ROC curve) of CD4⁺ T absolute count is 0.566, and the cut-off value is 469. In CKD patients, the CD4⁺ T absolute count of 469 was the best cutoff, the sensitivity and specificity of predicting infection were 39.9% and 73.36%, respectively. The related data were briefly summarized in Table 3. It is well known that CD4⁺ T <200 cells/ μ l was always used as a cutoff value for predicting opportunistic infections in AIDS patients. Therefore, we compared the predictive value of CD4⁺ T <200 cells/ μ l and CD4⁺ T <469 cells/ μ l for infection in CKD patients. The AUC area for the comparison between CD4⁺ T <469 cells/ μ l and bacterial infections was 0.57 (95%CI 0.52 to 0.61, Fig3). The AUC area for the comparison between CD4⁺ T <200 cells/ μ l and bacterial infection was 0.51 (95%CI 0.46 to 0.56, Fig3). Therefore, for bacterial infection, CD4⁺ T <469 cells/ μ l showed a better predictive value than CD4⁺ T <200 cells/ μ l [Table3][Fig3].

Discussion

Chronic kidney disease (CKD) is an important contributor to morbidity and mortality in non-communicable diseases. According to the latest data released by The Lancet, as of 2017, approximately 697.5 million people worldwide have been diagnosed with CKD¹⁰. In China, the prevalence rate is 10.8%, and the number of patients reached 132.3 million¹⁰. CKD is susceptible to infection, and the mortality rate significantly increased in CKD patients with infection^{11,12}. Therefore, CKD patients have been the subject of particular attention to prevent and treat the infection. In recent studies, some investigators indicated that infection was directly or indirectly associated with a disturbed immune response and account for the high incidence of morbidity and mortality among patients with kidney dysfunction. It is suggesting that prevention and intervention approaches for infection should be considered more actively. Although the mechanisms of immune dysfunction in chronic kidney disease have not been fully elucidated, cellular immune dysfunction may play a vital role in the pathogenesis of CKD and infection^{13,14}. In cellular immune, T lymphocytes are not only a major component of adaptive immunity but also a significant barrier to bacterial pathogen infection¹⁵. However, at present, the researches on the correlation between CKD T lymphocyte subgroup levels and infection are still rare. The opinions on the change of T lymphocyte in CKD patients are not uniform conclusions. In addition, there is little research in immune function of the T cells to evaluate and predict the risk of infection for patients with chronic kidney disease. Therefore, the present study focused on CKD patients and characteristics of T lymphocyte count, in order to find a new and feasible strategy to prevent infection in CKD patients.

Recent evidences suggested that renal insufficiency would affect the immune system. However, the previous studies only focused on ESRD or renal transplant patients, and the early stage of CKD patients were received less attention. In our study, we found that T lymphocyte levels gradually declined according with renal function decreases in CKD patients. It is well known that glucocorticoids and immunosuppressive agents could significantly affect the body's immune capacity. In order to exclude

these effects, we analyzed the patients in screening group. At last, same results were acquired in CKD and screening patients. It indicates that T lymphocyte levels in CKD patients may related to renal function.

With renal function decreased, many toxic substances which could be filtered by the healthy kidneys, would remain in the CKD patients body and lead to the development of the uremic syndrome. The accumulation of various toxic substances would destroy the function in immune regulation and contribute to the disorders of cellular immune function. Patients with advanced CKD and ESRD exhibited abnormal CD4⁺/ CD8⁺ ratios. Recent studies confirmed that the severity of azotemia, iron overload and oxidative stress would directly influence the apoptosis of the naive and central memory T cells of CD4⁺ and CD8⁺^{16,17}. In addition, the worse renal function, the malnutrition would more likely occur. A study of 5248 American adults with aged 60 years and over, found glomerular filtration rate less than 30 ml/min/1.73 m² was an independent factor for malnutrition¹⁸. The immune system consumes high amounts of energy, and strongly dependents on nutritional balance¹⁹. Therefore, the renal function, nutrition and immunity are closely linked. Based upon the interaction of all of these mentioned factors, T lymphocyte dysfunctions gradually increase according with the loss of renal function in CKD patients.

In our study, the absolute counts of CD3⁺, CD4⁺, and CD8⁺ T lymphocyte cells in the infected group of CKD patients are lower than those in the non-infected group. Especially, the infection rate in CD4⁺ T cells <410 cells/ μ l group was significantly higher than normal group with CD4⁺ T cells >410 cells/ μ l. This feature was not affected by hormones and immunosuppressant. We inferred the total number of lymphocyte subsets could reflect the immune status in CKD patients. T cells represent a significant component of the adaptive immune system and play a central part in cell-mediated immunity²⁰. When naive T cells expose to antigen, it start on clonal expansion, differentiation, generation of the memory T cells and effector T cells. Effector T cells perform their function via secretion of cytokines and destruction of target cells. CD3⁺ molecules are labeled on the surface of all differentiated mature T lymphocytes, and CD4⁺ T molecules are labeled on the helper T lymphocytes which were pivotal for the development of protective immune responses. CD4⁺ T cells could interact with antigen-presenting cells to direct the immune response, activate cytotoxic T cells and macrophages and promote the maturation of B cells into plasma cells to further produce antibodies. Furthermore, they could recruit immune cells such as polymorphonuclear leukocytes (PMN), eosinophils, basophils and so on., and enhance macrophages ability to promote the function of anti-inflammation. CD8⁺ T cells could destroy virally infected cells and tumor cells, and participate in transplant rejection²¹. Only T lymphocytes could recognize and respond to processed antigens through the C3b antigen-antibody complex. Therefore, a decrease of the T lymphocytes could reduce the ability to control the inflammatory, and CKD patients would be more susceptible to bacterial infection. In addition, as early stage CKD patients still have reduced lymphocyte counts, immunosuppressive therapies need a comprehensive evaluation.

In previously, some researches have been carried out on using T-lymphocyte counts to monitor patient's cellular immunity levels, but the study which adequately covers all CKD patients, was still rare. Most studies in this field of using CD4⁺ T cell counts to assess infection risk of patients, had only focused on

acquired immune deficiency syndrome (AIDS)^{22,23}. It is well known that the index of CD4⁺ T cells <200 cells/ μ l is always used as a cutoff value for predicting opportunistic infections in acquired immune deficiency syndrome (AIDS) patients. In our studies, the infection of CKD patients was closely related to the levels of T lymphocyte cells,, especially CD4⁺ T lymphocyte cells. In screening cases, CD4⁺ T lymphocytes may not be affected by related immune factors such as hormones, immunosuppressive agents or autoimmune diseases. The results remain consistent with CKD patients. CD4⁺ T<410 cells/ μ l was an independent influencing factor for infection in CKD patients and screening cases. Furthermore, we utilized receiver operating characteristic (ROC) curve analysis to determine a useful cut-off score. We found CD4⁺ T lymphocyte count of less than 469 cells/ μ l are at the highest risk of infection in CKD patients. Therefore, the predictive value of CD4⁺ T <200 cells/ μ l and CD4⁺ T <469 cells/ μ l for infection in CKD patients was compared. We found the sensitivity was obviously improved from 6.9% to 39.9% in our new cutoff in CKD patients. As we all know, infections in CKD patients are relatively hidden, but progress quickly, and significantly affect the prognosis. It is very important for CKD patients to early diagnose and treat the complication of infection. Our data provide a useful cutoff of CD4⁺ T <469 cells/ μ l. Although the sensitivity of cutoff value of CD4⁺ T <469 cells/ μ l remains to be improved, but for the early diagnosis and treatment of infection, it is still very important for physicians to improve the survival and quality of life in CKD patients. The finding provided a new direction for early clinical treatment of infection in CKD patients.

We all know that CD4⁺ T cells play a broad role in defending against pathogens. The interaction of cytokines and receptor-ligands stimulate naive CD4⁺ T cells to interact with dendritic cells in lymphoid tissues and further differentiate into T helper(Th), regulatory T cells (Treg), T follicular helper (Tfh) cells, etc. The above subgroups could secrete specific cytokines, such as IFN- γ , IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IL-17F, IL-21, IL-22, IL-24, lymphotoxin α , tumor necrosis factor α (TNF- α) and granulocyte macrophage colony-stimulating factor (GM-CSF), transforming growth factor (TGF)- β , to participate in innate and adaptive immune responses. It could be seen that the disorder of CD4⁺ T lymphocyte's function could not only reduce the body's immunity, lead to infection, but also affect humoral immunity, stimulate the production of cytokines, and further aggravate kidney damage. However, in our present study, due to the limitation of experimental conditions, we couldn't determine the counts of CD4⁺ T lymphocyte subgroups. The further studies are needed to clarify the function of CD4⁺ T lymphocyte subgroups in CKD patients.

Conclusions

In Summary, we collected and analyzed T lymphocytes from 447 patients with chronic kidney disease and found T lymphocyte counts decrease with kidney function declines. Infections in CKD patients were associated with reductions of T lymphocyte counts. Furthermore, CD4⁺ T absolute count was an independent influencing factor for infection. Our further study detected that CD4⁺ T <469 cells/ μ l was a useful indicator for predicting the occurrence of infection, and CD4⁺ T <469 cells/ μ l showed a better

sensitivity than CD4⁺ T <200 cells/μl. These results remind us to monitor the level of T lymphocytes in CKD patients and timely detect the decline in cellular immune function. Therefore, monitoring in T lymphocytes would become a new direction for infection prevention in CKD patients.

Abbreviations

AIDS: acquired immune deficiency syndrome

AUC area: area under the ROC curve

BD: Becton,Dickinson and Company

CD: cluster of differentiation

CI: confidence interval

CKD: chronic kidney disease

ESRD: end-stage renal disease

GFR: glomerular filtration rate

GM-CSF: granulocyte macrophage colony-stimulating factor

K/ DOQI: The National Kidney Foundation's Kidney Disease Outcomes Quality Initiative

OR: odds ratio

PCT: procalcitonin

ROC: Receiver Operating Characteristic

TGF: transforming growth factor

TNF-α: tumor necrosis factor α

Declarations

Ethics approval and consent to participate

Our study was conducted according to the tenets of the Declaration of Helsinki. The study protocol was approved by the Medical Ethics Committee of Inner Mongolia People's Hospital (Hohhot, China). All participants gave written informed consent in accordance with the Helsinki Declaration of 1975, revised in 2008.

Consent for publication

Informed written consent was obtained from all participants prior to participation in the study. Written informed consent was obtained from the patients for publication.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ZYL and JY collected the clinical information, interpreted the data and drafted the manuscript. YL and ZY supported the data collection, interpretation of the data, and writing of the manuscript. YL and DLN tested T-lymphocyte counts in CKD patients. YL and LGP reviewed the draft and made critical modifications. All authors read and approved the final manuscript.

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Tables

Table 1. Compared infection rate in CKD and screening patients with lymphocyte count below normal or normal levels

	Infection patients	Non-infected patients	Total	*p value
	n (%)	n (%)	n	
447 CKD patients				
CD3<690	44(51.8)	41(48.2)	85	0.236
CD3≥690	159(43.9)	203(56.1)	362	
CD4<410	71(55.0)	58(45.0)	129	0.012
CD4≥410	132(41.5)	186(58.5)	318	
CD8<190	26(59.1)	18(40.9)	44	0.078
CD8≥190	177(43.9)	226(56.1)	403	
165 screening patients				
CD3<690	23(69.7)	10(30.3)	33	0.022
CD3≥690	60(45.5)	72(54.5)	132	
CD4<410	27(73.0)	10(27)	37	0.003
CD4≥410	56(43.8)	72(56.2)	128	
CD8<190	15(75.0)	5(25)	20	0.034
CD8≥190	68(46.9)	77(53.15)	145	

Table 2. Independent risk factor associated with infection in 447 patients and screening cases.

	OR (95% CI)	P-value
447 CKD patients		
CD4 + T cell counts	1.58 [1.04-2.41]	0.03
eGFR	1.00 [0.99-1.00]	0.03
165 Selecting cases		
CD4 + T cell counts	3.47 [1.55-7.77]	0.01

CI: confidence interval.

OR: odds ratio.

Table 3. compared the predictive value of CD4⁺ T <200 cells/μl and CD4⁺ T <469 cells/μl for infection in CKD patients

Variable	AUC	Sensitivity%	Specificity%	p
CD4 ⁺ T <200 cells/μl	0.508	6.90	94.67	0.0056
CD4 ⁺ T <469 cells/μl	0.566	39.90	73.36	

AUC: Area under the ROC curve

Figures

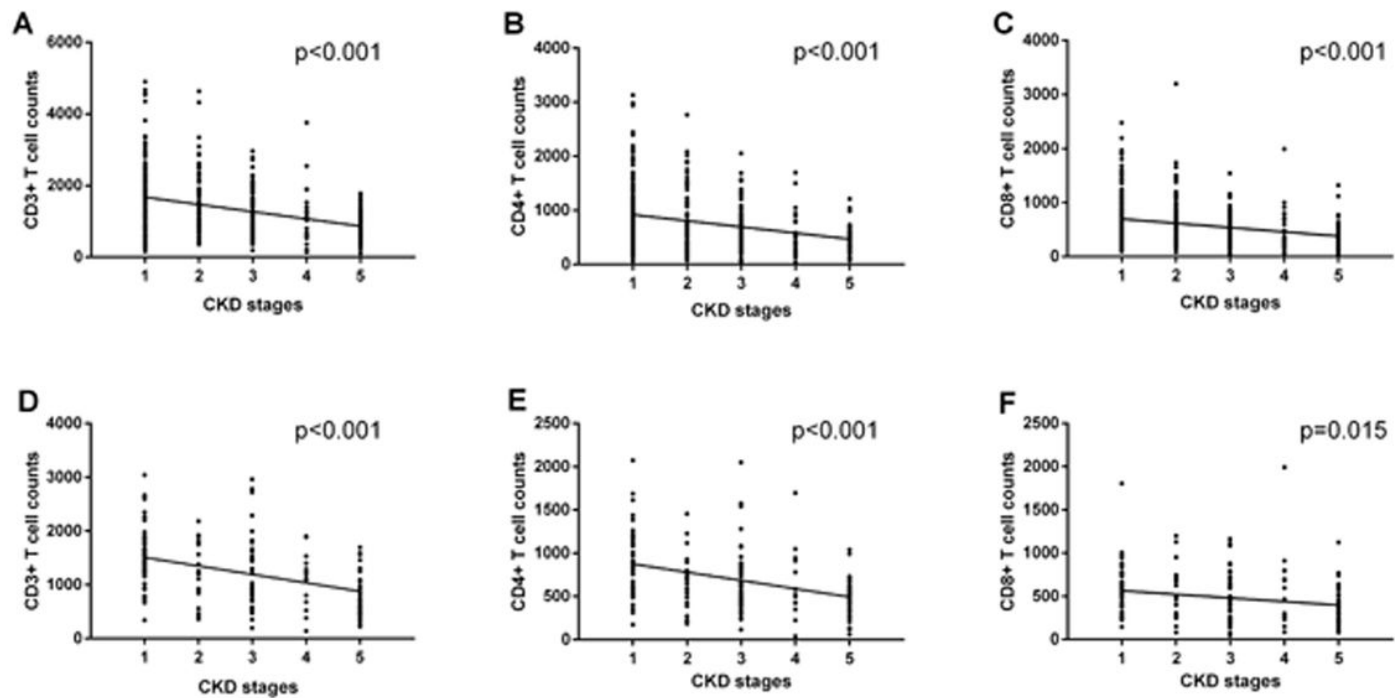


Figure 1

Correlation between CKD stage (X axis) and T lymphocyte count (Y axis) (A) With the decline of renal function, the absolute counts of CD3+ T lymphocytes gradually decreased in 447 patients ($P < 0.001$). (B) With the decline of renal function, the absolute counts of CD4+ T lymphocytes gradually decreased in 447 patients ($P < 0.001$). (C) With the decline of renal function, the absolute counts of CD8+ T lymphocytes gradually decreased in 447 patients ($P < 0.001$). (D) With the decline of renal function, the absolute counts of CD3+ T lymphocytes gradually decreased in screening patients ($P < 0.001$). (E) With the decline of renal function, the absolute counts of CD4+ T lymphocytes gradually decreased in screening patients ($P < 0.001$). (F) With the decline of renal function, the absolute counts of CD8+ T lymphocytes gradually decreased in screening patients ($P = 0.015$). "screening patients" means CKD patients with autoimmune diseases (systemic lupus erythematosus, vasculitis, etc.) or treating with hormones or immunosuppressants within 2 years were excluded;

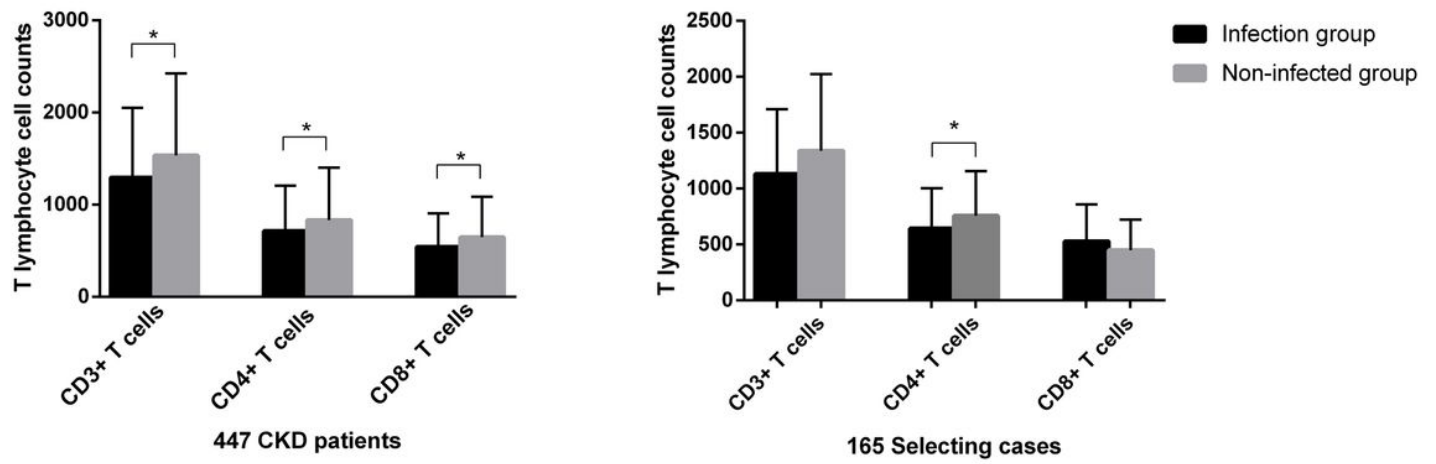


Figure 2

Comparison of absolute lymphocyte counts between infected and non-infected groups in 447 patients and screening cases. * means $p < 0.05$. "screening patients" means CKD patients with autoimmune diseases (systemic lupus erythematosus, vasculitis, etc.) or treating with hormones or immunosuppressants within 2 years were excluded.

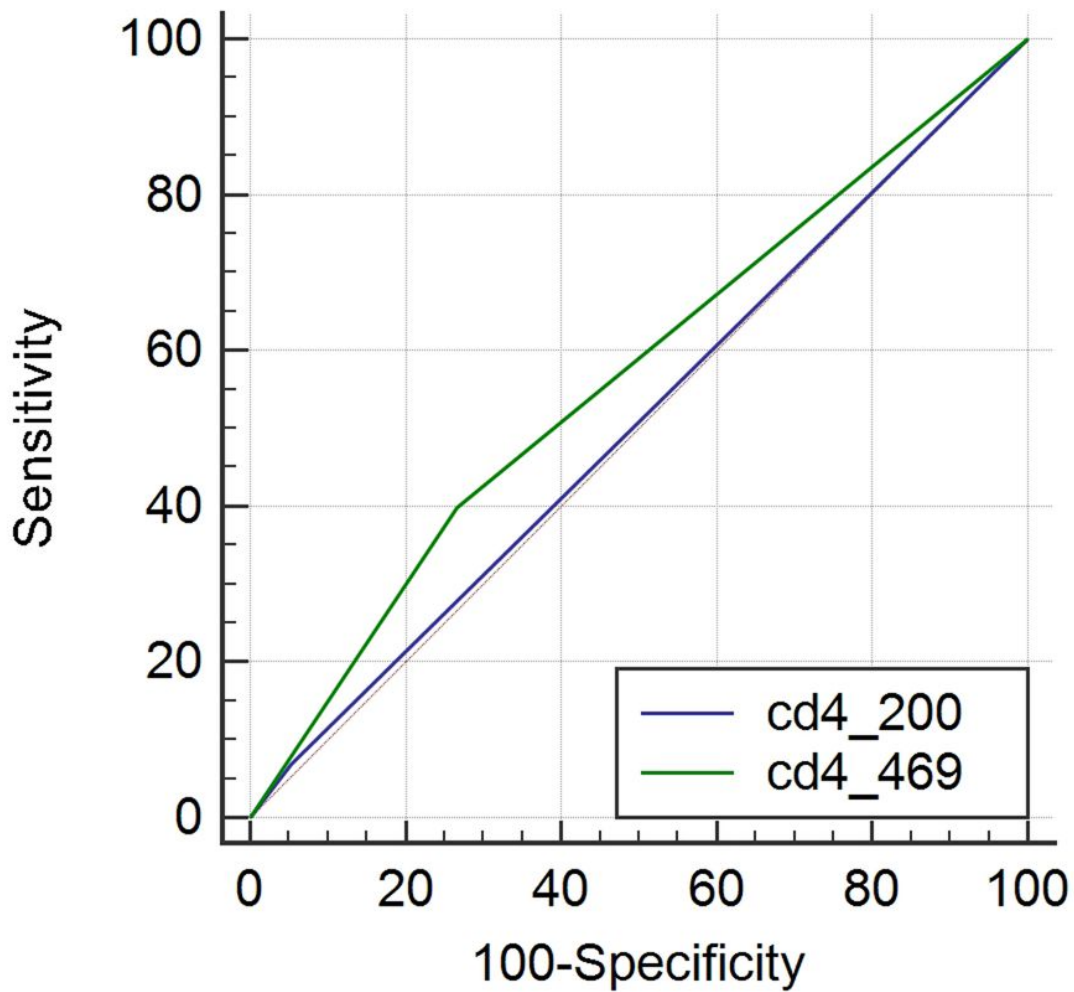


Figure 3

Receiver operating characteristic (ROC) curve for the absolute counts of CD4+ T lymphocytes to predict bacterial infection in CKD patients. The green solid line is the ROC curve for CD4+ T cells <469 cells/μl to predict bacterial infection in CKD patients. The area under the curve was 0.57 (95%CI 0.52 to 0.61). The blue solid line is the ROC curve for CD4+ T cells <200 cells/μl to predict bacterial infection in CKD patients. The area under the curve was 0.51 (95%CI 0.46 to 0.56).