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Disease activity prediction and prognosis of anti-GBM nephritis based on T lymphocyte subset ratios

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Abstract

Introduction: Cell-mediated autoimmunity, especially the autoreactivity of T cells, is known to underlie the initiation of anti-glomerular basement membrane disease. However, the T lymphocyte subsets that determine the disease activity, renal fibrosis, and prognosis of anti-GBM disease have not been clearly elucidated.

Methods: The T lymphocyte subsets (CD4+ and CD8+) were examined on peripheral blood and renal biopsy tissues from 65 patients with biopsy proven anti-GBM disease. Patients were divided into the high ratio group and low ratio group according to the cutoff values in the receiver operating characteristic curve analysis. The correlations of T lymphocyte subsets with clinical, pathological data, and renal outcome were analyzed.

Results: By the end of follow-up, 45 patients (69.2%) developed end-stage renal disease (ESRD). In peripheral blood, the CD4+/CD8+ ratio showed a predictive ability with a sensitivity and specificity of 91.3% and 52.9%, respectively, which gave rise to a cutoff value of 0.89. There was a significant difference in the activity index between these two groups (3.91 ± 1.38 vs. 2.89 ± 1.13, $p = 0.007$). In the renal tissues, the CD4+/CD8+ ratio had the optimal cutoff point of 0.82 with a sensitivity of 57.8% and specificity of 85%. The renal activity index was higher for the renal tissues with high CD4+/CD8+ ratios than that of tissues with low CD4+/CD8+ ratios (4.32 ± 1.55 vs. 3.37 ± 1.41, $p = 0.016$). Peripheral blood CD4+/CD8+ ratios of ≥0.89 or renal tissue CD4+/CD8+ ratios of < 0.82 positively correlated with poor renal prognosis in patients with anti-GBM nephritis.

Conclusions: The CD4+/CD8+ ratio was associated with renal activity index both in peripheral blood and renal tissue and predicts the renal prognosis of patients with anti-GBM nephritis.

Keywords

Anti-GBM nephritis, immune cells, disease activity, renal outcome

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Introduction

Anti-glomerular basement membrane (anti-GBM) disease is a rapidly progressive and often life-threatening autoimmune disorder,[1] with an estimated incidence of 1–2 cases per million population per year.[2] The disease usually manifests rapidly progressive glomerulonephritis (RPGN) and pulmonary hemorrhage as those in Goodpasture syndrome. In most patients, renal lesions are characterized by glomerular fibrinoid necrosis and crescent formation.[3] The renal outcome of both of Goodpasture syndrome and anti-GBM nephritis is poor.

With the discovery of anti-GBM antibodies and their pathogenicity, plasma exchange and immunosuppressive

agents have become the primary treatments for this disease.[4] In spite of these treatments, the disease still deteriorates rapidly and approximately 55% of the patients require dialysis at onset.[5,6] It has been reported that renal survival rates are between 20% and 40% and that the renal outcome is related to the severity of crescent formation on kidney biopsy according to a study of one-year follow-up.[7,8]

Although the pathogenic role of anti-GBM autoantibodies against the noncollagenous domain 1 of α3 chain of type IV collagen (α3(IV)NC1) has been fully confirmed,[9] there is strong evidence that the full development of this disease involves cell-mediated autoimmunity, especially autoreactivity of T cells. The antigen-specific CD4+ T cells per se could initiate kidney damage in animal model, even in the absence of anti-GBM antibodies.[10] In rodent models of the disease which is induced by heterologous anti-GBM globulin, the absence of CD4+ T cell in the effector phase of the disease can effectively prevent the recruitment of glomerular macrophage and crescentic formation.[11] Therefore, the CD4+ T cell response mainly affects the initiation of immune effector mechanisms and resultant patterns of glomerular injury.[12-14] On the other hand, the presence of CD8+ T cells in patients and some experimental models of crescentic glomerular nephritis (GN) raises the prospect of T cell–mediated cytotoxicity as an effector mechanism of injury. However, early injury is likely to reflect inflammatory cell infiltration and activation rather than cytotoxicity. This effect is traditionally attributed to CD4+ T helper cells, which recruit and activate macrophages by the delayed-type hypersensitivity mechanism.[11] Therefore, the CD4/CD8 T cell ratio may determine which immune activation prevails.

In recent years, there has been increasing evidence that both CD4+ and CD8+ T cells are involved in anti-GBM experimental models; however, their contributions to the disease development in patients remain unclear. In the present work, to characterize the T cells in anti-GBM diseases and determine whether the T cell subsets could predict disease activity and prognosis in patients, we investigated the subtypes of T cells in peripheral blood and renal biopsy tissues from patients with anti-GBM RPGN and determined their relationship with clinical and pathological data, as well as renal outcome.

Methods and materials

Study population

A total of 65 patients who had biopsy-proven anti-GBM nephritis and diagnosed between August 2004 and August 2017 in the National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing University School of Medicine, were included in this retrospective study. All patients were used to establish a model and cutoff value of CD4+/CD8+ ratio. The high CD4+/CD8+ ratio group and the low CD4+/ CD8+ ratio group were fitted as categories. The clinical, laboratory, and pathological data were compared between the two groups. This work was approved by the Institutional Review Board of Jinling Hospital, and we received informed consent from all the patients.

Clinical and laboratory measurements

Clinical data, including the patients' general condition, duration of disease, clinical manifestations, and relevant laboratory tests were collected from medical records. Dialysis dependency at onset referred to the need for urgent renal replacement therapy (RRT) on the first admission. The outcome of end-stage renal disease (ESRD) was defined as estimated glomerular filtration rate (eGFR) ≤15 mL/min/1.73 m^2, dialysis dependency for more than 3 months during follow-up, or kidney transplantation.[15]

Biopsy specimen evaluation

Renal biopsy specimens were investigated by two pathologists from national clinical research center of kidney diseases individually, who were blinded by the patients' clinical data and prognosis information. Each specimen had at least 10 glomeruli per slide at the time of biopsy. The routine biopsy examination included HE, PAS, Masson's trichrome, and PASM staining. In order to assess the activity and chronicity of the lesions, serial 1 μm sections were stained with silver methenamine and counterstained with hematoxylin eosin. Routine paraffin sections were immunostained using a four PAP method to determine CD4+, CD8+ T cells in the interstitium and glomeruli. The cells' density was calculated by counting the total number of target cells, for example, CD4+ cells, confined within each of 20 random 0.25 mm^2 fields (each of these field was delineated by a 1 cm^2) ocular grid that was viewed at ×400

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magnification), and the result was expressed at the average value per mm2. Pathological classification was performed referring to the literature.[16] Interstitial lesions, such as interstitial infiltration, interstitial fibrosis, and tubular atrophy, were semi-quantitatively graded on a scale of 0–3 (0, 0%; 1, 0–25%; 2, 25–50%; 3, >50%), and fibrinoid necrosis was scored as absent or present (0 or 1).

For calculation of activity index (modified from[17]), the cellular crescents were quantitatively graded in a range of 0–3, in which 0 point stands for absence; 1 point for less than 25% of glomeruli involved; 2 points for 25%–50%; and 3 points for greater than 50%. Additionally, the grades of interstitial inflammation and fibrinoid necrosis were included in the activity index, resulting in a maximum score of 7. According to disease activity, we divided the patients into mild (score of 0–2), moderate (score 3–5), and severe (score 6–7) groups. The chronicity index (modified from[17]) included the percentage of fibrous crescents, grade of interstitial fibrosis, and tubular atrophy, resulting in a maximum chronicity score of 9. Similarly, we also divided patients into mild (score of 0–2), moderate (score 3–5), and severe (score 6–9) groups.

Sample preparation and flow cytometry

One hundred microliters of peripheral blood were collected and analyzed using the BD FACS Aria flow cytometer. Antibodies used are Biolegend FITC anti-human CD4 and Biolegend PerCp anti-human CD8. In addition, 10^3–10^5 PBMC were added to specific primary antibodies at the optimal concentration. The sample was incubated in a dark room for 20 min. After washing twice, the secondary antibody, fluorochrome-conjugated anti-human immunoglobulin anti-human (FITC anti-human), and PerCp were added to cells, followed by incubation on ice in a dark room for 15–20 min and then washing. Data acquired from the flow cytometer were analyzed using BD FACS diva software. The lymphocyte population was gated and then the lymphocyte subsets were gated: CD3 + CD4 + helper T cells and CD3 + CD8 + cytotoxic T cells.

Statistical analysis

All date were analyzed by using SPSS 22.0 statistical software IBM (NY, USA). Normally distributed measurement data were represented as mean ± SD and were compared between groups using the t test. Non-normally distributed measurement data were expressed in median and interquartile range (P25 and P75), and differences were assessed with the rank-sum test. Categorical variables were expressed as percentages (%), and X^2 test was used for comparison between groups. The cutoff values of CD4+/CD8+ ratio were determined using receiver operating characteristic (ROC) curve analysis, and the dependent variable was the occurrence of ESRD. Correlations between considered parameters were assessed using Spearman's R test. Cox multifactor regression analysis was used to analyze the relationship between all risk factors and the life span of kidneys. The survival rate was analyzed using a Kaplan–Meier survival curve. A difference of $p < 0.05$ was considered statistically significant.

Results

General conditions and clinical features

Of 65 patients with anti-GBM nephritis, 38 (58.5%) were men and 27 (41.5%) were women, with a median age of 43 (range, 25–55) years at biopsy. The median interval between onset of the disease and kidney biopsy was 45 (range, 30–70) days. More than half of the patients had oliguria/anuria (55.4%), gross hematuria (53.8%), and hypertension (53.8%). Most of the patients (80%) required dialysis at presentation. The patients had massive microscopic hematuria (median, $950*10^4$/ml) and moderate degree of proteinuria (median, 1.85 g/24 h). The mean CD4/CD8 T cell ratios in peripheral blood and renal tissues are 1.59 ± 0.94 and 0.94 ± 0.26, respectively (Table 1). By the end of follow-up, 45 patients (69.2%) progressed to ESRD.

Table 1. Baseline characteristics of the 65 patients with anti-GBM disease.

	Patients
Variable	*N* = 65
Age, years	43 (25, 55)
Male, %	38 (58.5)
Duration of symptoms[a], d	45 (30, 70)
Hemoptysis, %	22 (33.8)
Oliguria/anuria, %	36 (55.4)
Dialysis dependent at presentation, %	53 (81.5)
Urinary protein (g/24 h)	1.85 (0.96, 6.39)
Microscopic hematuria (10^4/mL)	950 (410, 1600)
Hemoglobin (g/L)	84.8 ± 18.6
SCr (mg/dL)	5.40 ± 3.58
Anti-GBM level (RU/ml)	156.5 ± 87.1
CD4/CD8 ratio in peripheral blood	1.59 ± 0.94
CD4/CD8 ratio in renal tissues	0.94 ± 0.26
Cellular crescents, %	23.8 (23.8, 40.0)
Sclerotic glomeruli, %	29.6 (20.0, 29.6)
Plasma exchange	26 (40.0)
Prednisone	57 (87.7)
Cyclophosphamide	26 (40.0)

[a]The median interval between onset of the disease and kidney biopsy.

Clinicopathologic features of CD4/CD8 subgroups in peripheral blood

ROC curve was prepared to evaluate the predictive ability of ESRD for the immune cells in the cohort of 65 cases. As indicated in Figure 1, CD4+/CD8+ ratio in peripheral blood showed 91.3% sensitivity and 52.9% specificity. The sensitivity and specificity of CD4+/CD8+ ratio were maximized at the optimal cutoff value of 0.89. Compared with the low CD4+/CD8+ ratio group, the patients in high CD4+/CD8+ ratio group were older (45 vs. 29; $p = 0.022$). Besides, the patients in the high ratio group presented a significantly higher proportion of oliguria/anuria (66.0 vs. 27.8%; $p = 0.006$) and a higher percentage of dialysis at presentation (89.4 vs. 55.6%; $p = 0.002$; Table 2).

At baseline, there was no significant difference in serum creatinine (SCr) between the two groups. The patients in high CD4+/CD8+ ratio group had higher levels of microscopic hematuria (1200 vs. $462*10^4$/mL, $p = 0.041$). The level of circulating anti-GBM antibodies was significantly higher in the high ratio group than that in the low ratio group (175.0 ± 79.8 vs. 99.4 ± 86.5 g/L, $p = 0.005$).

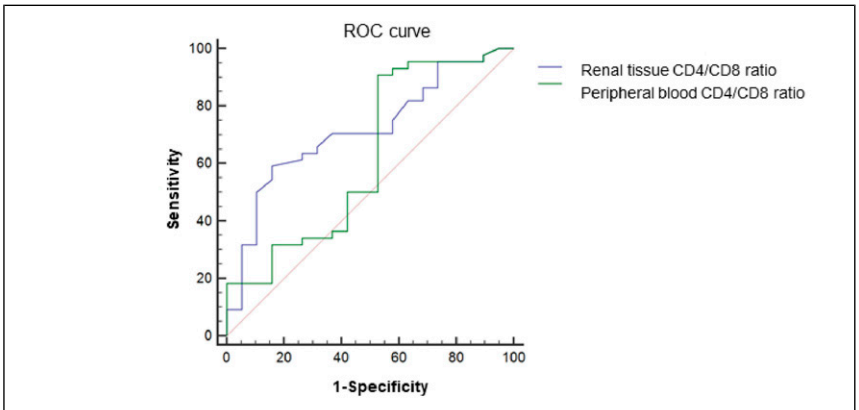
Percutaneous renal biopsies were performed under ultrasonography guidance, and the pathological data were reviewed independently by two experienced nephropathologists. In the high CD4+/CD8+ ratio group, the proportion of cellular crescents were higher than that in the low ratio group (median 24.4 vs. 4.8%, $p = 0.032$), and there were also more inflammatory cells infiltrated the renal interstitium (2.36 ± 0.64 vs. 1.83 ± 0.62; $p = 0.004$). Interstitial fibrosis and tubular atrophy were not significantly different between the two groups. There was a marked difference in the activity index between the two groups with more evidence of acute damage in the high CD4+/CD8+ ratio group (3.91 ± 1.38 vs. 2.89 ± 1.13, $p = 0.007$; Table 2).

Histopathologic characteristics of CD4/CD8 subgroups in renal tissues

For the CD4+/CD8+ ratio in renal tissues, the optimal cutoff point was 0.82, with a low sensitivity of 57.8% and high specificity of 85% [area under the curve (AUC): 0.72, 95% CI: 0.59–0.82; Figure 1]. The duration of symptoms prior to receiving renal biopsy was 33 days in the high CD4+/CD8+ ratio group, and this was significantly shorter than that in the low ratio group (median 52 days; $p = 0.047$). Glomerulosclerosis appeared more severe in the low ratio group (median: 25% vs. 9.1%; $p = 0.04$). Renal activity index was higher for the high CD4+/CD8+ ratio group in renal tissues (4.32 ± 1.55 vs. 3.37 ± 1.41, $p = 0.016$), while the chronicity index was lower in the group compared with that for the low ratio group (1.95 ± 1.13 vs. 3.74 ± 1.83, $p = 0.00$; Table 3). The distribution of CD4+ and CD8+ T cells in kidney of the patients with anti-GBM nephritis was shown in Figure 2.

Correlation analysis

The ratio of peripheral and renal CD4+/CD8+ T cells correlated significantly with activity index (r = 0.405, $p = 0.001$; r = 0.429, $p = 0.000$) and chronicity index (r = −0.280, $p = 0.024$; r = −0.253, $p = 0.042$). We also observed that the ratio of CD4+/CD8+ cells was low for patients with a high renal chronicity index. However, the patients with a high disease activity had a significantly higher ratio of CD4+/CD8+ T cells than the patients with mild and moderate disease activities (Figure 3).



	ROC-AUC (CI%)	P	Cut-off (sensitivity, specificity)
Renal tissue CD4/CD8 ratio	0.72(0.59, 0.82)	0.002	0.82 (57.8%, 85.0%)
Peripheral blood CD4/CD8 ratio	0.62 (0.49, 0.74)	0.050	0.89 (91.3%, 52.9%)

Figure 1. Receiver operating characteristic area under the curve (ROC-AUC) for prediction of ESRD in anti-GBM nephritis patients.

Table 2. General conditions and clinicopathologic features of CD4/CD8 subgroups in peripheral blood.

	CD4/CD8 ≥ 0.89	CD4/CD8 < 0.89	
	N = 47	*N* = 18	*p*
Baseline			
Age, years	45 (26, 58)	29 (24, 45)	0.022
Male, %	25 (53.2)	13 (72.2)	0.164
Duration of symptoms, d	44 (28, 65)	60 (33, 80)	0.389
Hemoptysis, %	14 (29.8)	8 (44.4)	0.264
Oliguria/anuria, %	31 (66)	5 (27.8)	0.006
Hypertension, %	28 (59.6)	7 (38.9)	0.134
Pulmonary infection	15 (31.9)	5 (27.8)	0.746
Dialysis dependent at presentation, %	42 (89.4)	10 (55.6)	0.002
Laboratory data			
Urinary protein (g/24 h)	1.44 (0.75, 3.37)	3.91 (1.62, 6.83)	0.088
Microscopic hematuria (10⁴/mL)	1200 (560, 2200)	462 (250, 1163)	0.041
Hemoglobin (g/L)	84.7 ± 16.1	85.0 ± 24.5	0.964
SCr (mg/dL)	5.13 ± 3.31	6.12 ± 4.24	0.352
Serum albumin (g/L)	32.6 ± 5.3	30.7 ± 5.2	0.185
IgG (g/L)	11.6 ± 5.1	7.3 ± 3.9	0.007
Anti-GBM level (RU/ml)	175.0 ± 79.8	99.4 ± 86.5	0.005
Histopathologic characteristics			
Normal glomeruli, %	2.7 (0, 14.5)	8.3 (0, 32.5)	0.142
Crescents, %	72.1 (58.4, 98.1)	55.2 (33.7, 84.3)	0.091
Cellular crescents, %	24.4 (6.9, 42.8)	4.8 (0, 30.8)	0.032
Fibrous/fibrocellular crescents, %	38.1 (17.9, 69.3)	39.3 (0, 69.2)	0.703
Sclerotic glomeruli, %	18.6 (0, 33.3)	25.9 (10.2, 68.5)	0.092
Interstitial fibrosis	1.43 ± 0.83	1.50 ± 0.99	0.759
Tubular atrophy	1.19 ± 0.97	1.17 ± 0.86	0.924
Interstitial infiltrate	2.36 ± 0.64	1.83 ± 0.62	0.004
Tubulitis	28 (59.6)	10 (55.6)	0.769
Treatment			
Plasma exchange	20 (42.6)	6 (33.3)	0.497
Prednisone	42 (89.4)	15 (83.3)	0.810
Cyclophosphamide	15 (31.9)	11 (61.1)	0.032

Table 3. Histopathologic characteristics of CD4/CD8 subgroup in renal tissues.

	CD4/CD8 ≥ 0.82	CD4/CD8 < 0.82	
Characteristic	*N* = 22	*N* = 43	*p*
Duration of symptoms, d	33 (23, 66)	52 (35, 71)	0.047
Crescents, %	73.5 (55.3, 100)	69.2 (47.6, 88.5)	0.369
Cellular crescents, %	24.5 (8.5, 55.2)	12.5 (0, 36.3)	0.123
Fibrous/fibrocellular crescents, %	24.2 (6.8, 71.4)	43.8 (20.6, 69.2)	0.370
Globally sclerotic glomeruli, %	9.1 (0, 24.1)	25 (11.5, 67.7)	0.004
Interstitial fibrosis	1.36 ± 0.90	1.58 ± 0.91	0.362
Tubular atrophy	0.82 ± 0.80	1.42 ± 0.96	0.014
Interstitial infiltrate	2.23 ± 0.75	2.26 ± 0.66	0.875
Tubulitis	13 (59.1)	25 (58.1)	0.941
Activity index	4.32 ± 1.55	3.37 ± 1.41	0.016
Chronicity index	1.95 ± 1.13	3.74 ± 1.83	0.000

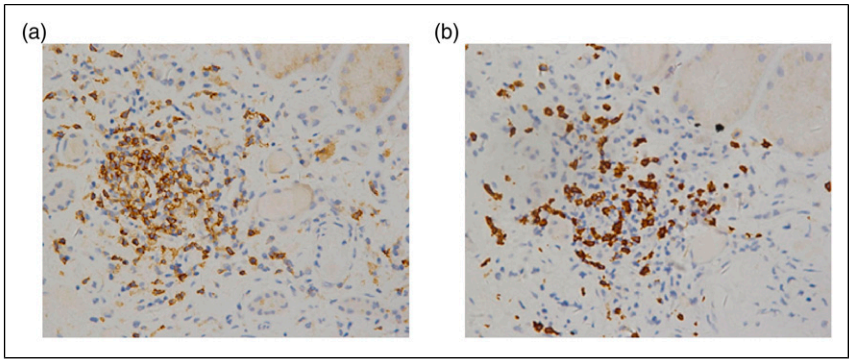


Figure 2. Distribution of T lymphocyte subsets in patients with anti-GBM nephritis. Immunohistochemical (IHC) staining showing CD4 (a) and CD8 (b) expression in the kidney of patients with anti-GBM nephritis (IH, ×400).

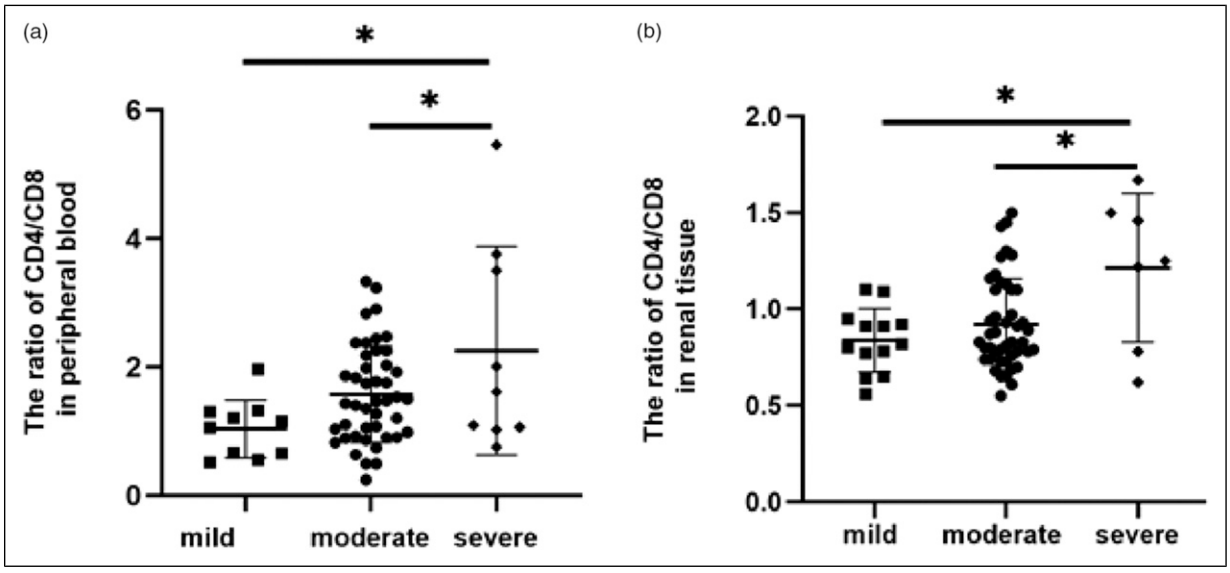


Figure 3. CD4/CD8 ratio for different levels of disease activity. Wilcoxon rank-sum test with Bonferroni correction was used to assess the differences among the groups. There is a significant difference in the peripheral blood CD4/CD8 ratio between the low and the high activities and between the moderate and high activities of anti-GBM nephritis (a); such differences are also observed in renal tissue (b).

Follow-up and outcome

By the end of follow-up, the 1-year and 5-year cumulative renal survival rates were 25.7% and 18.5%, respectively. The patient cumulative survival rates were 94.6%, 89%, and 66.1% in 1, 3, and 10 years, respectively. Oliguria/anuria, SCr, anti-GBM level, urine NAG, dialysis dependency at onset, the ratio of CD4+/CD8+, percentage of crescents, and activity index were related to ESRD in univariable Cox regression analyses (Table 4). We added significant variables including oliguria/anuria, serum creatinine, anti- GBM level, urine NAG, the ratio of CD4+/CD8+ (in peripheral blood and renal tissue), percentage of crescents, and activity index to the multivariate Cox regression analysis and found that the ratio of CD4+/CD8+ in peripheral blood and renal tissue was significantly associated with ESRD (HR = 4.382, $p = 0.045$; HR = 0.277, $p = 0.003$; Table 4). Further, we developed three models to examine the relationship between the CD4+/CD8+ ratio (in peripheral blood and renal tissue) and ESRD. The adjusted variables in each model were shown under Table 5. In the three models, the CD4+/CD8+ ratios in peripheral blood and renal tissues were still significant predictors of ESRD. It demonstrated that these parameters are independent risk factors for ESRD. In Kaplan–Meier analysis, the peripheral blood CD4+/CD8+ ≥0.89 or renal tissue CD4+/CD8+ < 0.82 positively correlated with poor long-term renal prognosis (Figure 4).

Table 4. Cox regression analysis of the independent risk factors for ESRD.

	Univariate analysis		Multivariate analysis	
Characteristic	HR (95% CI)	*p*	HR (95% CI)	*p*
Age, year	1.009 (0.992, 1.027)	0.325	—	—
Males	1.261 (0.744, 2.232)	0.441	—	—
Hemoptysis	0.924 (0.550, 1.723)	0.803	—	—
Oliguria/anuria	3.116 (1.453, 4.859)	0.001	—	—
Hypertension	1.643 (1.073, 3.379)	0.110	—	—
SCr (mg/dL)	1.105 (1.035, 1.203)	0.027	1.143 (1.002, 1.304)	0.046
Hemoglobin (g/L)	0.994 (0.987, 1.009)	0.303	—	—
anti-GBM level (RU/ml)	1.008 (1.001, 1.011)	0.004	—	—
Urinary protein (g/24 h)	1.015 (0.939, 1.139)	0.781	—	—
Urine NAG (u/g* cr)	1.010 (1.002, 1.018)	0.010	—	—
Dialysis dependency at onset	2.591 (1.099, 6.110)	0.030	—	—
Peripheral blood CD4/CD8 ≥ 0.89	2.591 (1.160, 4.754)	0.022	4.382 (1.418, 13.542)	0.045
<0.89	Ref	—	—	—
Renal tissues CD4/CD8 ≥ 0.82	0.493 (0.361, 1.103)	0.020	0.277 (0.120, 0.642)	0.003
<0.82	Ref	—	—	—
Crescents (%) ≥ 50%	2.258 (1.210, 4.208)	0.049	—	—
<50%	Ref	—	—	—
Globally sclerotic glomeruli (%)	1.025 (0.990,1.063)	0.166	—	—
Activity index	1.255 (1.011, 1.535)	0.043	—	—
Chronicity index	0.988 (0.877, 1.130)	0.876	—	—

Table 5. Association between the CD4/CD8 ratio (in peripheral blood and renal tissue) and ESRD.

Model		HR (95% confidence interval)	*p* value
Model 1	Peripheral blood CD4/CD8 ≥ 0.89 vs. <0.89	4.382 (1.418, 13.542)	0.045
	Renal tissues CD4/CD8 ≥ 0.82 vs. <0.82	0.277 (0.120, 0.642)	0.003
Model 2	Peripheral blood CD4/CD8 ≥ 0.89 vs. <0.89	3.094 (1.375, 6.960)	0.006
	Renal tissues CD4/CD8 ≥ 0.82 vs. <0.82	0.470 (0.237, 0.933)	0.031
Model 3	Peripheral blood CD4/CD8 ≥ 0.89 vs. <0.89	3.127 (1.223, 8.001)	0.017
	Renal tissues CD4/CD8 ≥ 0.82 vs. <0.82	0.486 (0.236, 1.002)	0.051

Model 1: adjusted by oliguria/anuria, serum creatinine, anti-GBM level, urine NAG, the ratio of CD4+/CD8+ (in peripheral blood and renal tissue), percentage of crescents, and activity index.
Model 2: adjusted by oliguria/anuria, dialysis dependency at onset, anti-GBM level, urine NAG, the ratio of CD4+/CD8+ (in peripheral blood and renal tissue), percentage of crescents, and activity index.
Model 3: adjusted by serum creatinine, the ratio of CD4+/CD8+ (in peripheral blood and renal tissue), anti-GBM, and cyclophosphamide treatment.

Discussion

Anti-GBM glomerulonephritis has been considered as a typical example for autoantibody-mediated autoimmune disease. Recent studies have shown that T cell–mediated mechanism is involved in not only the glomerular damage but also the anti-GBM antibody responses to multiple GBM antigens.[18] Early histological observations showed that antigen-specific CD4+ T cells themselves could initiate glomerular injury.[19] A single nephritogenic T cell epitope alone is sufficient to induce the clinical manifestations of anti-GBM glomerulonephritis, including proteinuria, hematuria, and acute renal injury. However, the specific impact of CD4+ and CD8+ cells on the development of anti-GBM glomerulonephritis and the potential relationship between these two T cell subsets in the process remain to be elucidated.

The CD4+/CD8+ ratio has emerged as a novel biomarker for immunodeficiency disease. Increasing evidence suggests a CD4+/CD8+ ratio >1 as the cutoff to define normal values in the general population. In the present study, we found a marked peripheral blood and renal interstitial accumulation of CD4+ and CD8+ T cells in anti-GBM nephritis and performed the first detailed

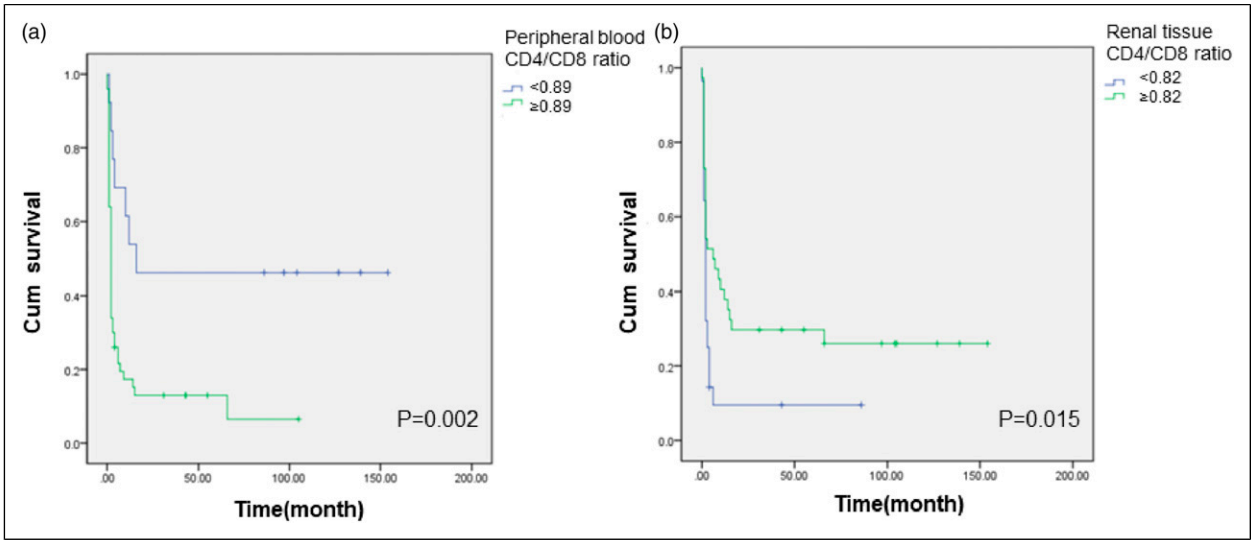


Figure 4. CD4/CD8 ratio and renal prognosis in anti-GBM nephritis patients. (a) Renal prognosis was significantly poorer in anti-GBM nephritis patients with higher peripheral blood CD4/CD8 ratio (≥0.89), $p = 0.002$; (b) renal prognosis was significantly poorer in anti-GBM nephritis patients with lower renal tissue CD4/CD8 ratio (<0.82), $p = 0.015$.

investigation of the relationship between the CD4+/CD8+ ratio and disease activity. We found that when CD4+ cells are predominant over CD8+ cells in the peripheral blood and kidney tissues, the patients are usually at the early stage of disease. This observation was consistent with the study by Reynolds J et al.[20] Our study also showed that the ratio of CD4+/CD8+ correlated positively with the disease activity index. These results may represent early pathological changes of anti-GBM nephritis and suggest that CD4+ cells are mainly involved in acute kidney injury.[21] However, the ratio of peripheral and renal CD4+/CD8+ T cells had negative relativity with chronicity index. These data mean that lower CD4+/CD8+ T cell ratio indicates more chronic lesions. Therefore, the peripheral blood CD4+/CD8+ ratio helps in the assessment of disease activity and choice of treatment regimen for patients if their renal biopsy is not available.

The 5-year renal survival rate in this study was 18.5%, which was lower than that reported by Levy et al.[22] but in line with that by Cui et al.[7] Severe infection is a common early complication and very important for prognosis in anti-GBM disease.[23] In our study, the incidence of lung infection was 31%, which was lower than Caillard et al.'s study.[23] There's no serious infection reported in the course of disease, besides at disease onset or after aggressive therapy. It also has been reported that for patients with clinical features, such as high SCr, oliguria, or anuria, a delayed diagnosis results in a poor renal prognosis.[22,24,25] Wu et al.[26] showed that mast cells infiltration was associated with chronic lesions in anti-GBM nephritis and may be involved in the loss of renal function. According to our data, the patients with more severe CD4+ T cells infiltration may be at the acute stage of the disease. We considered and balanced the baseline characteristics of patients for oliguria/anuria, SCr, anti-GBM level, urine NAG, the ratio of CD4+/CD8+, percentage of crescents, and activity index. The CD4+/CD8+ ratio of either peripheral blood or renal tissue was an independent predictor for more rapid progression of anti-GBM GN, which frequently leads to dialysis or ESRD.

Moreover, the distribution of T lymphocyte subsets in renal tissue and peripheral blood is not parallel. We found that the peripheral blood CD4+/CD8+ ratio ≥ 0.89 or renal tissue CD4+/CD8+ ratio < 0.82 correlated with ESRD, suggesting that the kidney prognosis would be poor when the peripheral blood is predominated by CD4+ cells or the kidney tissue is predominated by CD8+ cells infiltration. Therefore, we think that lymphocyte subsets in peripheral blood and renal tissue may participate in the pathogenesis of anti-GBM GN through different mechanisms. It has been shown that infiltration of T lymphocytes in renal tissue is more important than activation of T cells in peripheral blood in the development of anti-GBM nephritis and that CD8+ cells in kidney tissue play a crucial role for the progression of anti-GBM nephritis in an early phase. Some experimental studies have indicated that CD8+ cells and the soluble factor secreted by them might directly and/or indirectly promote tissue damage.[27] In childhood IgAN, Watanabe et al.[28] showed that the number of glomerular CD8+ T cells was the most sensitive predictor of disease progression.

The combination therapy (plasma exchange, corticosteroids, and cyclophosphamide) has a generally favorable effect on both disease symptoms and renal survival of all anti-GBM patients. However, sixty percent of our patients did not receive the combination therapy and the possible reasons were long duration of oliguria/anuria, low likelihood of renal function recovery, or the high cost. In general, for patients with renal-limited disease with a SCr ≥ 600 μmol/L or with ≥80% crescent formation, they rarely benefit from such therapy.[29-31] In a word, despite the need for more controllable data, the value of intensive treatment for patients with anti-GBM disease and advanced kidney disease seems very limited.

This study has some limitations, such as the nature of retrospective observation and an inevitable selection bias. Other limitations include the small sample size of cohort due to the rareness of the disease and the nature of single-center study with Chinese patients. Therefore, a larger, multicenter study nationwide or worldwide might be needed to verify the current results. Moreover, differential treatment of the patients was a potential confounder in our study. It would be necessary to conduct histopathological examination on patients with anti-GBM nephritis who are treated similarly.

Conclusion

Cellular immunity may play a vital role in the inflammatory kidney injury of anti-GBM patients. Our study has shown that CD4+/CD8+ ratio is associated with renal activity index in anti-GBM nephritis. In addition, the peripheral blood CD4+/CD8+ ≥0.89 or renal tissue CD4+/CD8+ <0.82 is the strong predictor of renal survival. This knowledge may be helpful for the diagnosis, prognosis, and treatment of anti-GBM nephritis.

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Declaration of conflicting interests

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Ethical approval

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Informed consent

Written informed consent was obtained from all the subjects before the study.

References

1. Jennette JC, Falk RJ, Bacon PA, et al. (2013) 2012 revised international chapel hill consensus conference nomenclature of vasculitides. *Arthritis Rheum* 65: 1–11
2. Canney M, O'Hara PV, McEvoy CM, et al. (2016) Spatial and temporal clustering of anti-glomerular basement membrane disease. *Clin J Am Soc Nephrol* 11: 1392–1399.
3. Jennette JC (2003) Rapidly progressive crescentic glomerulonephritis. *Kidney Int* 63: 1164–1177.
4. Lerner RA, Glassock RJ and Dixon FJ (1967) The role of anti-glomerular basement membrane antibody in the pathogenesis of human glomerulonephritis. *J Exp Med* 126: 989–1004.
5. Levy JB, Turner AN, Rees AJ, et al. (2001) Long-term outcome of anti-glomerular basement membrane antibody disease treated with plasma exchange and immunosuppression. *Ann Intern Med* 134: 1033–1042.
6. Cui Z, Zhao J, Jia XY, et al. (2011) Antiglomerular basement membrane disease: Outcomes of different therapeutic regimens in a large single-center Chinese cohort study. *Medicine (Baltim)* 90: 303–311.
7. Huart A, JosseAG, Chauveau D, et al. (2016) French Society of Hemapheresis: Outcomes of patients with Goodpasture syndrome: A nationwide cohort-based study from the French society of hemapheresis. *J Autoimmun* 73: 24–29.
8. Herody M, BobrieG, Gouarin C, et al. (1993) Anti-GBM disease: Predictive value of clinical, histological and serologicaldata. *Clin Nephrol* 40: 249–255.
9. Lerner RA, Glassock RJ and Dixon FJ (1999) The role of anti-glomerular basement membrane antibody in the pathogenesis of human glomerulonephritis. *J Am Soc Nephrol* 10: 1389–1404.
10. Wu J, Hicks J, Borillo J, et al. (2002) CD4(+) T cells specific to a glomerular basement membrane antigen mediate glomerulonephritis. *J Clin Invest*. 109: 517–524.
11. Huang XR, Tipping PG, Apostolopoulos J, et al. (1997) Mechanisms of T cell-induced glomerular injury in anti-glomerular basement membrane (GBM) glomerulonephritis in rats. *Clin Exp Immunol* 109: 134–142.
12. Huang XR, Holdsworth SR and Tipping PG (1994) Evidence for delayed- type hypersensitivity mechanisms in glomerular crescent formation. *Kidney Int* 46: 69–78.

13. Tipping PG, Huang XR, Qi M, et al. (1998) Crescentic glomerulonephritis in CD4- and CD8-deficient mice. Requirement for CD4 but not CD8 cells. *Am J Pathol* 152: 1541–1548.
14. Li S, Holdsworth SR and Tipping PG (1997) Antibody independent crescentic glomerulonephritis in mu chain deficient mice. *Kidney Int* 51: 672–678.
15. Korbet SM, Lewis EJ, Schwartz MM, et al. (2000) Factors predictive of outcome in severe lupus nephritis. *Am J Kidney Dis*. 35:904–914.
16. Berden AE, Ferrario F, Hagen EC, et al. (2010) Histopathologic classification of ANCA-associated glomerulonephritis. *J Am Soc Nephrol* 21: 1628–1636.
17. Franssen CF, Gans RO, Arends B, et al. (1995) Differences between anti-myeloperoxidase- and anti-proteinase 3-associated renal disease. *Kidney Int* 47:193–199.
18. Tipping PG and Holdsworth SR (2006) T Cells in crescentic glomerulonephritis. *J Am Soc Nephrol* 17: 1253–1263.
19. Lou YH (2004) Anti-GBM glomerulonephritis: A T cell-mediated autoimmune disease? *Arch Immunol Ther Exp* 52(2):96–103.
20. Reynolds J, Moss J, Smith J, et al. (1999) Ultrastructural abnormalities of kidney and lung in an animal model of Goodpasture's disease. *Kidney Int* 55: 2557.
21. Paust HJ, Turner JE and Riedel JH (2012) Chemokines play a critical role in the cross-regulation of Th1 and Th17 immune responses in murine crescentic glomerulonephritis. *Kidney Int* 82, 72–83.
22. Levy JB, Turner AN, Rees AJ, et al. (2001) Long-term outcome of anti-glomerular basement membrane antibody disease treated with plasma exchange and immunosuppression. *Ann Intern Med*134: 1033–1042.
23. Caillard P, Vigneau C, Halimi JM, et al. (2020) Severe Infection in anti-glomerular basement membrane disease: A retrospective multicenter French study. *J Clin Med* 9(3): 698–712.
24. Daly C, Conlon PJ, Medwar W, et al. (1996) Characteristics and outcome of anti-glomerular basement membrane disease: A single-center experience. *Ren Fail*. 18: 105–112.
25. Jindal KK (1999) Management of idiopathic crescentic and diffuse proliferative glomerulonephritis: Evidence-based recommendations. *Kidney Int Suppl*. 70: S33–S40.
26. Wu XM, Zhang YY, Zhang MC, et al. (2016) Significance of mast cell renal infiltration in patients with anti-GBM nephritis. *Ren Fail*.38(6):906–913.
27. Carter LL and Dutton RW (1996) Type 1 and type 2: A fundamental dichotomy for all T-cell subsets. *Curr Opin Immunol* 8:336–342.
28. Chen A, Lee K, D'Agati VD, et al. (2018) Bowman's capsule provides a protective niche for podocytes from cytotoxic CD8+ T cells. *J Clin Invest*.128(8): 3413–3424.
29. Merkel F, Pullig O, Marx M, et al. (1994) Course and prognosis of anti-basement membrane antibody (anti-BM-Ab)-mediated disease: report of 35 cases. *Nephrol Dial Transplant*.9: 372–376.
30. Flores JC, Taube D, Savage CO, et al. (1986) Clinical and immunological evolution of oligoanuric anti-GBM nephritis treated by haemodialysis. *Lancet*. 1: 5–8.
31. Alchi B, Griffiths M, Sivalingam M, et al. (2015) Predictors of renal and patient outcomes in anti-GBM disease: Clinicopathologic analysis of a two-centre cohort. *Nephrol Dial Transplant*. 30(5): 814–821.