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Anti-carbonic anhydrase II antibody reflects urinary acidification defect especially in proximal renal tubules in patients with primary Sjögren syndrome

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Abstract

Primary Sjögren syndrome (pSS) is a systemic autoimmue disease featured by excessive autoantibody production. It has been demonstrated that anti-carbonic anhydrase II (anti-CA II) antibody is correlated with renal tubular acidosis in pSS; however, no further details about urinary acidification defect have been reported, and the antibody's relationship with other organ impairments remains unknown. This case-control study aimed to examine anti-CA II antibody levels in relation to various systemic complications in pSS, and evaluate its potential role as a organ-specific biomarker in a Chinese cohort.

Serum anti-CA II antibody levels were determined using ELISA in 123 patients with pSS and 72 healthy controls. The medical records of the patients were collected, and the correlation between serum anti-CA II antibody and clinical/immunological parameters was investigated.

Serum anti-CA II antibody level and its positive rate were significantly increased in pSS patients compared with controls, and ANA-positive patients presented even higher titers of the antibody. In anti-CA II positive group, remarkably higher urine pH and bicarbonate, as well as lower urine titratable acid and serum potassium were observed, which indicated renal tubular acidification dysfunction both involving bicarbonate reabsorption and acid secretion. In addition, platelet count and complement 3, complement 4 levels decreased, whereas serum IgG, IgA and γ-globulin levels increased notably in accord with a higher EULAR SS disease activity index score in these patients. Further analysis showed that anti-CA II antibody was most elevated in patients with defect in bicarbonate reabsorption, reflecting proximal renal tubular injury, rather than in patients with distal renal tubular acidosis as previously reported.

In conclusion, anti-CA II antibody reflects renal (especially proximal renal tubular) and hematologic impairment as well as increased disease activity in pSS. It may act as a serum biomarker of systemic damage of pSS.

Abbreviations: anti-CA II = anti-carbonic anhydrase II, C3 = complement 3, ESSDAI = EULAR SS disease activity index, HCs = healthy controls, pSS = primary Sjögren syndrome, RTA = renal tubular acidosis, TA = titratable acid.

Keywords: anti-carbonic anhydrase II antibody, Sjögren syndrome, thrombocytopenia, urinary acidification defect

1. Introduction

Primary Sjögren syndrome (pSS) is an autoimmune disease characterized by chronic inflammation of exocrine glands as well as systemic impairment involving multiple organs.[1] Among them, renal tubular acidosis (RTA) and thrombocytopenia are two frequently encountered complications.[1,2] And if not discerned and treated timely, these patients may develop severe electrolyte disturbance and fatal hemorrhage, which could be urgernt and life-threatening.

As is known, the pathogenisis of pSS is highly associated with abnormal B cell activation and excessive autoantibody production, and anti-SSA/Ro, anti-SSB/La antibodies and ANA are widely applied as serum biomarkers in pSS diagnosis.[1] However, unfortunately these antibodies lack organ specificity, failing to reflect and prognose organ-specific damages. Therefore, other biomarkers are needed for precise evaluation of systemic impairment.

Carbonic anhydrase II (CA II), found in the cytosol of vast human somatic cells including erythrocytes and renal tubule

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cells, is an essential basic metalloenzyme that catalyzes the reversible hydration of carbon dioxide to generate a proton and a bicarbonate ion in acid-base homeostasis regulation in vivo.[3] Previous studies suggested that the autoantibody against CA II is associated with RTA in pSS patients and animal models.[3–5] Yet these studies only focused on distal RTA (dRTA), and there's no further reports specifying the reabsorption of bicarbonate and excretion of titratable acid (TA) of urinary acidification defect in detail. Besides, no research of anti-carbonic anhydrase II (anti-CA II) antibody with systemic damage other than kidneys in pSS has been carried out.

This case-control study aims to elucidate the relationship between anti-CA II antibody and various systemic impairments in pSS and evaluate its potential role as an organ-specific serum biomarker by analyzing the clinical and laboratory parameters of pSS patients with high anti-CA II antibody level.

2. Methods

2.1. Patients and samples

This study included 123 consecutive pSS patients admitted to Peking University People's Hospital from December 2016 to June 2018, all of whom fulfilled the 2016 ACR/EULAR criteria for pSS,[6] and patients with other diseases (such as diabetes, hypertention and autoimmune diseases including SLE) were excluded for potential renal involvement. Seventy-two healthy controls (HCs) were selected from age- and sex-matched, disease-free subjects who underwent routine medical examinations.

All the subjects provided written informed consent before the clinical/laboratory data and peripheral blood samples were obtained. The study was approved by the Medical Ethics Committee of Peking University People's Hospital, and performed according to the Declaration of Helsinki.

2.2. ELISA assay

The levels of serum anti–CA II antibody were measured by ELISA as previously described using erythrocyte CA II (Sigma, St. Louis, Missouri) as a ligand[3]; absorbance (O.D. value) was determined at 450 nm and expressed in ELISA units per milliliter (EU/mL). Anti-CA II was considered positive above a threshold of mean + 2 × S.D. of the HC group.

2.3. Clinical and laboratory evaluation

Clinical manifestations and complications as well as laboratory data including complete blood count, electrolyte and renal function parameters were reviewed retrospectively from medical records of pSS patients. Urinary acidification defect was evaluated by fasting urine pH, bicarbonate levels, TA and NH_4^+, whereas dRTA was diagnosed if the arterial bicarbonate level was < 22 mmol/L at baseline and the fasting urine pH was > 5.5.[4]

All the patients underwent extensive immunological analysis for the detection of antibodies, including ANA (indirect immunofluorescence), anti-SSA/SSB (double immunodiffusion), rheumatoid factor-IgM (rate nephelometry) and anti-α-fodrin antibody (enzyme-linked immunosorbent assay). All the detections were performed by standard commercial kits according to the manufacturer's instructions. Serological features such as ESR, CRP, immunoglobulins, complement 3 (C3) and complement 4 levels were also collected. Finally, the EULAR SS Disease Activity Index (ESSDAI) score was calculated.[7]

2.4. Statistical analysis

Comparisons were tested for statistical significance using the Student *t* test or the Mann–Whitney *U* test or the chi-square test, as appropriate. Spearman rank correlation test was employed in order to correlate patients' parameters with anti-CA II levels. SPSS version 16.0 (SPSS, Chicago, IL) was used for statistical analysis. *P*-values < .05 were considered statistically significant.

3. Results

3.1. Serum anti-CA II antibody was increased in pSS patients

As in Table 1, most pSS patients were female (96.7%), and median age was 55 years old (IQR 50–63), with median disease duration as 8 years (IQR 4–14). Among all the patients, 54 (43.9%) were positive for anti-CA II. Figure 1A and B show that both the median levels (0.877 vs 0.794 EU/mL, $P < .001$) and positive rates (43.90 vs 4.17%, $P < .001$) of serum anti-CA II antibody in pSS patients were siginificantly increased compared with HCs. And patients with positive ANA presented notably higher levels of anti-CA II antibody ($P < .05$), whereas the other autoantibodies (anti-SSA, anti-SSB and rheumatoid factor) made no difference in anti-CA II levels (Fig. 1C–F).

3.2. pSS patients with anti-CA II antibody were more often suffered from urinary acidification defect as well as thrombocytopenia

We devided the pSS patients into anti-CA II (+) and (-) groups depending on their antibody levels and compared various clinical manifestations and complications of them. Therein, urinary acidification defect was defined as abnormality in fasting urine pH, bicarbonate, TA or NH_4^+ levels.

Out of 123 patients, only 27 (21.95%) were diagnosed with dRTA, while up to 55 (44.7%) had got urinary acidification defect, and 35 (28.46%) had thrombocytopenia. As shown in Table 2, among all the systemic complications, patients with anti-CA II antibody were particularly more often suffered from urinary acidification defect and thrombocytopenia (90.7 vs 8.7%, 40.7 vs 18.8%, respectively, $P < .01$). Other clinical characteristics presented no difference in 2 groups.

Subsequently, we investigated blood and urine test parameters in 2 groups of pSS patients (Table 1). Likewise, in anti-CA II positive group, increased urine pH and decreased platelet count were observed (6.44 vs 6.23, $P < .05$, 141.9 vs 190.9×10^9/L, $P < .01$, respectively). As for electrolyte, urine bicarbonate was elevated (19.11 vs 13.33mmol/L, $P < .01$), whereas urine TA (4.29 vs 8.74mmol/L, $P < .05$) and serum potassium levels (3.58 vs 3.77, $P < .05$) were lower, which indicated renal tubular acidification defect both involving bicarbonate reabsorption and acid excretion. In Spearman correlation test, anti-CA II antibody showed positive correlations with fasting urine pH and bicabonate, and negative correlations with platelet, serum potassium and urine TA (Table 3).

3.3. pSS patients with anti-CA II antibody had marked hyperglobulinemia and hypocomplementemia in consistent with higher disease acitivities

Table 1 reveals that serum IgG, IgA and γ-globulin levels were all increased (18.5 vs 16.5g/L, $P = .001$, 3.70 vs 2.89g/L, $P < .05$, 25.5 vs 19.9%, $P = .001$, respectively), while C3, complement 4 decreased (0.819 vs 0.961g/L, $P = .001$, 0.165 vs 0.203g/L, $P < .01$, respectively) markedly in anti-CA II (+) group. Meanwhile, these patients also presented higher ESSDAI scores (5 vs 4, $P < .01$), indicating higher disease acitivities. The Spearman correlation test coincided with the result above, demonstrating anti-CA II antibody's positive correlations with immunoglobulin, ESSDAI and negative correlations with complements (Table 3).

Table 1

Clinical and laboratory characteristics of pSS patients and comparison after stratification on anti-CA II positivity.

Index	All pSS patients (n = 123)	Anti-CAII (+) (n = 54)	Anti-CAII (–) (n = 69)	*P* value
General information				
Female	119 (96.7)	51 (94.4)	68 (98.9)	.319
Age (yr)	55.0 (50.0–63.0)	54.5 (47.8–64.3)	55.0 (51.0–61.0)	.986
Disease duration (yr)	8.0 (4.0–14.0)	7.1 (4.0–15.3)	8.0 (4.0–13.3)	.659
Blood test parameters				
WCC (10^9/L)	4.3 (3.2–6.6)	4.6 (3.3–6.8)	4.2 (3.1–6.4)	.207
Hemoglobin (g/L)	117.0 (105.0–127.0)	116.5 (100.6–129.3)	117.0 (106.0–125.5)	.762
Platelet (10^9/L)	169.4 ± 86.9	141.9 ± 85.8	190.9 ± 82.1	.002
Creatine (μmol/L)	58.0 (51.0–73.0)	56.0 (50.0–74.3)	61.0 (51.5–72.5)	.347
Na (mmol/L)	142.0 ± 2.8	141.8 ± 2.1	142.1 ± 3.3	.608
K (mmol/L)	3.69 ± 0.44	3.58 ± 0.47	3.77 ± 0.40	.017
Cl (mmol/L)	108.0 ± 3.4	108.5 ± 3.3	107.6 ± 3.4	.120
CO_2CP (mmol/L)	23.9 ± 3.1	23.9 ± 3.5	23.8 ± 2.7	.833
Urine test parameters				
Fasting urine pH	6.32 ± 0.55	6.44 ± 0.58	6.23 ± 0.50	.041
Bicarbonate (mmol/L)	15.49 (10.83–21.30)	19.11 (12.46–22.71)	13.33 (9.17–17.78)	.004
TA (mmol/L)	7.45 (3.12–11.90)	4.29 (2.12–11.67)	8.74 (4.11–12.18)	.016
NH_4^+ (mmol/L)	22.56 (18.28–35.67)	22.32 (17.71–35.70)	23.58 (19.58–35.75)	.469
RBP (μg/L)	0.29 (0.05–0.66)	0.30 (0.01–0.69)	0.29 (0.14–0.63)	.609
β2-MG (μg/L)	460.0 (95.0–920.0)	527.5 (0.01–1000.0)	365.0 (135.0–668.0)	.552
NAG (U/L)	8.70 (2.75–12.99)	8.30 (0.01–13.20)	8.95 (4.40–12.90)	.134
24h urine protein (g/d)	0.09 (0.06–0.23)	0.09 (0.07–0.20)	0.09 (0.05–0.33)	.434
Immunological indexes and disease activity score				
CRP (mg/L)	2.98 (1.80–6.21)	2.68 (1.74–7.31)	3.00 (1.83–5.62)	.862
ESR (mm/h)	29.0 (15.0–49.0)	30.0 (16.8–53.0)	27.0 (13.5–44.0)	.278
IgG (g/L)	17.3 (12.2–24.5)	18.5 (16.4–25.5)	16.5 (10.5–20.9)	.001
IgA (g/L)	3.23 (2.03–5.00)	3.70 (2.87–5.08)	2.89 (1.70–4.59)	.019
IgM (g/L)	1.07 (0.71-1.68)	1.02 (0.75–1.54)	1.08 (0.70–1.98)	.472
Complement 3 (g/L)	0.912 (0.740–1.040)	0.819 (0.614–0.985)	0.961 (0.808–1.120)	.001
Complement 4 (g/L)	0.186 ± 0.074	0.165 ± 0.058	0.203 ± 0.080	.004
γ-globulin (%)	24.0 (18.1–28.7)	25.5 (21.4–32.3)	19.9 (15.5–27.5)	.001
RF (IU/ml)	36.0 (10.0–330.0)	42.9 (10.0–384.3)	26.3 (10.0–314.0)	.279
ANA	113 (91.9)	53 (98.2)	60 (87.0)	.055
Anti-SSA	96 (78.1)	42 (77.8)	54 (78.3)	.949
Anti-SSB	38 (30.9)	16 (29.6)	22 (31.9)	.788
Anti-α-fodrin (U/ml)	5.806 (2.923–12.292)	6.614 (3.038–15.781)	4.585 (2.638–10.084)	.141
ESSDAI score	5 (3–6)	5 (4–6)	4 (2–6)	.002

The data was expressed as mean ± S.D., or median (IQR), or n (%) as appropriate.

anti-CA II = anti-carbonic anhydrase II, ANA = antinuclear antibody, CO_2CP = carbon dioxide combining power, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, ESSDAI = the EULAR Sjögren Syndrome Disease Activity Index, IgA = immunoglobulin A, IgG = immunoglobulin G, IgM = immunoglobulin M, NAG = N-acetyl-β-D-glucosaminidase, pSS = primary Sjögren syndrome, RBP = retinol binding protein, RF = rheumatoid factor, TA = titratable acid, WCC = white cell count, β2-MG = β2-microglobulin.

3.4. pSS patients with dRTA were primarily impaired in function of renal acid excretion in distal tubules and glomeruli

To clarify the relationship of dRTA and anti-CA II antibody, we also stratified pSS patients depending on dRTA and comparison was done (Table 4). Patients with dRTA have significantly higher fasting urine pH and lower TA and serum potassium. C3 was decreased and ESSDAI score was increased in these patients, indicating higher disease acitivities. Nevertheless, different from anti-CA II (+) patients, dRTA patients' urinary acidification defect mainly manifested as TA and NH_4^+ excretion dysfunction in distal renal tubules, instead of bicarbonate reabsorption dysfunction in anti-CA II (+) patients. High Cl and low CO_2CP in serum were exhibited as well. Meanwhile, indexes regarding renal glomerular and tubular injury (including creatine, 24 hours urine protein, RBP and β2-MG) were harmed. These suggested that anti-CA II antibody was not simply related to typical dRTA as previously reported.

3.5. Anti-CA II antibody was most elevated in pSS patients with bicarbonate reabsorption defect

Finally, we focused on the different types of urinary acidification defect. Based upon patients' urinary acidification test, pSS patients were further subgrouped accordingly into 4 groups: normal acidification function, abnormal TA excretion (H^+ defect) only, abnormal bicarbonate reabsorption (HCO_3^- defect) only, both dysfunctions (H^+ & HCO_3^- defect). This grouping principle referred to the classification of RTA.[8]

As shown in Figure 1G and H, pSS patients with normal acidification function and HCs have comparable anti-CA II antibody levels and positive rates. But patients with urinary acidification defect presented significantly higher anti-CA II antibody ($P <$ .001), especially those with abnormal bicarbonate reabsorption.

4. Discussion

Excessive autoantibody production is prominent in the development of pSS, and a number of autoantibodies have emerged and been used as serum biomarkers in clinical practice.[9] However, there still lack organ-specific antibodies to guide us in recognizing and prognosing systemic complications. Anti-CA II antibody was first reported in sera of SLE and SS patients in the early 1990s independently by 2 research groups.[10,11] From then on, more studies regarding its role in various autoimmune conditions (such as type 1 diabetes, Grave disease and autoimmune pancreatitis[12–14]) were carried on, and anti-CA II antibody was shown to be related to RTA in pSS patients.[3,4] Further animal researches demonstrated

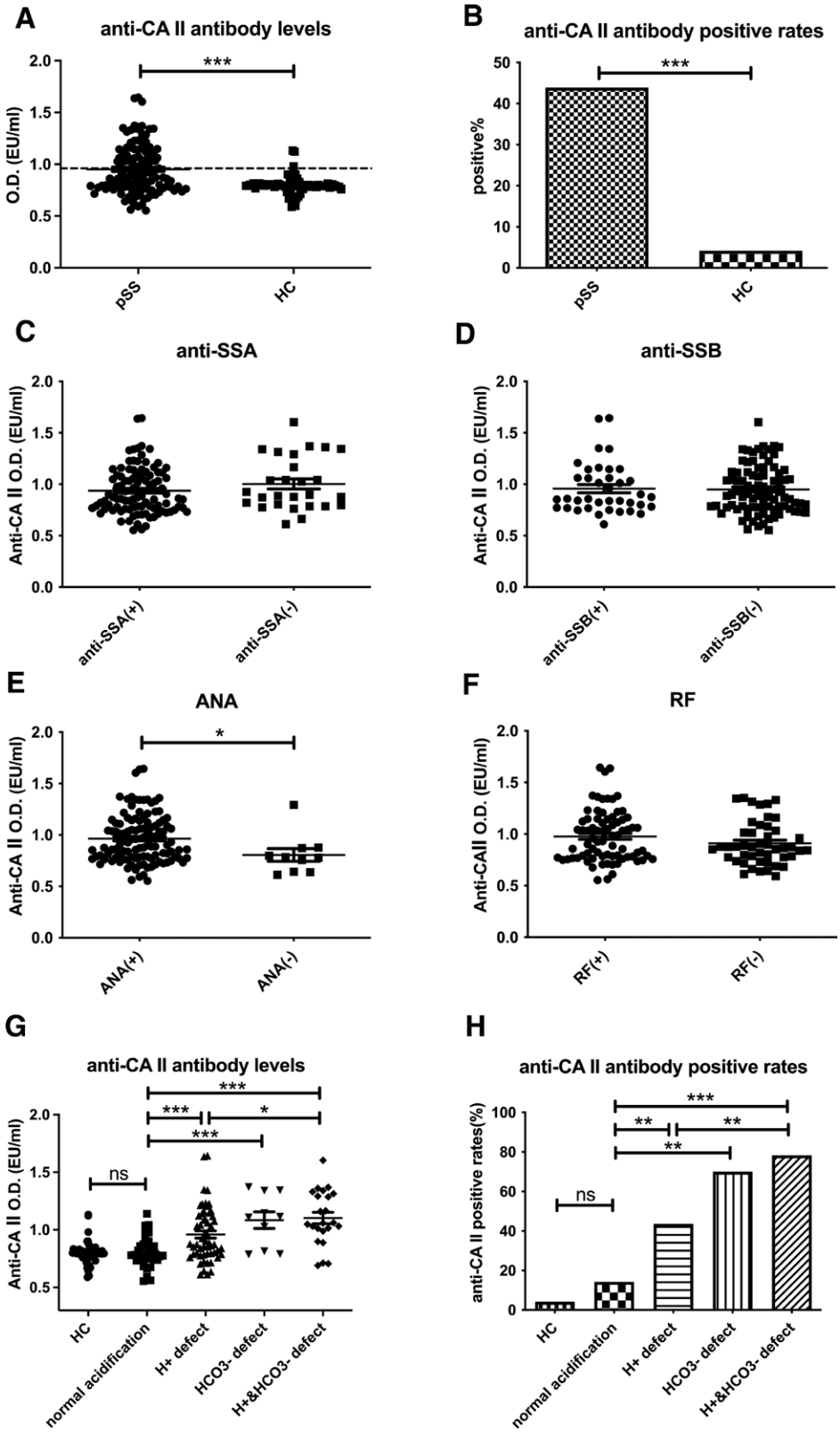

Figure 1. Comparison of anti-CA II in different subgroups of pSS with HC. (A and B) Comparison of anti-CA II antibody levels and its positive rates in pSS with controls. The dotted line indicates mean absorbance + 2 × S.D. of the HC as threshold for positivity. (C–F) Comparison of anti-CA II antibody levels in pSS patients with different autoantibodies. (G and H) Comparison of anti-CA II antibody levels and positive rates in pSS patients with different types of urinary acidification defect. **P* value < .05. ***P* value < .01. ****P* value < .001. Anti-CA II = anti-carbonic anhydrase II, pSS = primary Sjögren syndrome.

that CA II immunization could induce a pSS mouse model with RTA,[5,15] confirming the role of CA II antigen in the pathogenisis of pSS.

It is known that in pSS patients, the renal defecit often manifests as interstitial kidney injury, mainly involving distal tubules, rather than proximal tubules.[2,16] Thus dRTA is

Table 2

Comparison of clinical manifestations and complications of pSS patients after stratification on anti-CA II positivity.

Characteristics	Anti-CAII (+) (n = 54)	Anti-CAII (–) (n = 69)	*P* value
Xerophthalmia	47 (87.0)	61 (88.4)	.818
Xerostomia	52 (96.3)	69 (100.0)	.191
Rampant dental caries	37 (68.5)	41 (59.4)	.299
Glandular swelling	12 (22.2)	23 (33.3)	.175
Fever	15 (27.8)	10 (14.5)	.215
Arthralgia	23 (42.6)	31 (44.9)	.796
Rash	4 (7.4)	4 (5.8)	.729
Purpura	11 (20.4)	11 (15.9)	.377
Raynaud phenomenon	3 (5.6)	5 (7.3)	1.000
Lymphadenitis	4 (7.4)	7 (10.1)	.834
ILD	24 (44.4)	31 (44.9)	.957
dRTA	14 (25.9)	13 (18.8)	.346
Urinary acidification defect	49 (90.7)	6 (8.7)	<.001
Leucopenia	18 (33.3)	24 (34.8)	.866
Anemia	23 (42.6)	28 (40.6)	.630
Thrombocytopenia	22 (40.7)	13 (18.8)	.008
Peripheral neuropathy	5 (9.3)	4 (5.8)	.702
Hypothyroidism	9 (16.7)	8 (11.6)	.350
Autoimmune liver disease	5 (9.3)	14 (20.3)	.137

The data was expressed as n (%).

Anti-CA II = anti-carbonic anhydrase II, dRTA = distal renal tubular acidosis, ILD = interstitial lung disease, pSS = primary Sjögren syndrome.

Table 3

Correlation of anti-CA II antibody with clinical/laboratory parameters.

Parameters	*r*	*P* value
Age	0.062	.493
Disease duration	0.050	.582
WCC	0.208	.021
Hemoglobin	0.079	.385
Platelet	-0.189	.036
Creatine	-0.106	.243
Na	-0.010	.910
K	-0.194	.031
Cl	0.091	.319
CO_2CP	-0.010	.909
Fasting urine pH	0.221	.014
Urine bicarbonate	0.232	.010
Urine TA	-0.201	.026
Urine NH_4^+	0.018	.840
RBP	-0.051	.580
β2-MG	0.075	.412
NAG	-0.037	.686
24h urine protein	0.155	.208
CRP	0.017	.859
ESR	0.124	.172
IgG	0.328	<.001
IgA	0.191	.035
IgM	0.083	.360
Complement 3	-0.321	<.001
Complement 4	-0.225	.012
γ-globulin	0.432	<.001
RF	0.114	.210
Anti-α-fodrin	0.145	.127
ESSDAI score	0.268	.003

Anti-CA II = anti-carbonic anhydrase II, CO_2CP = carbon dioxide combining power, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, ESSDAI = the EULAR Sjögren Syndrome Disease Activity Index, IgA = immunoglobulin A, IgG = immunoglobulin G, IgM = immunoglobulin M, NAG = N-acetyl-β-D-glucosaminidase, RBP = retinol binding protein, RF = rheumatoid factor, TA = titratable acid, WCC = white cell count, β2-MG = β2-microglobulin.

believed to be the most common form of renal impairment in pSS, and all the previous studies about anti-CA II antibody concentrated on its correlation with dRTA, regardless of dysfunction in other parts.

To our knowledge, this is the first study to elucidate the correlation of anti-CA II antibody with urinary acidification dysfunction in detail. Distinct from previous researches, our study highlighted the concept of urinary acidification defect evaluated by urine acidification test, which is more sensitive and convenient to suggest the slight acid-base imbalance of the kidneys in the early stage. In our cohort, nearly half of the patients had urinary acidification defect to some extent. Furthermore, although patients with anti-CA II antibody manifested some features of dRTA (moderate dysfunction in acid excretion), they were fundamentally characterized by predominant dysfunction of bicarbonate reabsorption in proximal renal tubules. Moreover, other parameters related to dRTA such as serum Cl and urine $NH4^+$ were not affected either. This indicates that anti-CA II antibody reflects particularlly the damage in bicarbonic reabsoption function in proximal tubules, which contradicts common concepts. In this regard, we hypothesize the explanation by the physiological role of CA II. CA II distributed in renal tubules plays a key role in catalyzing the reaction: $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$.[3] Since anti-CA II antibody has been proven to react with CA II,[17] the catalyzing function of CA II may be damaged, hence leading to acidification defect involving $HCO_3^- + H^+$, rather than NH_4^+. This reminds us to pay more attention to proximal renal tubule dysfunction in pSS patients and supplement bicarbonate timely when a high titer of anti-CA II antibody is encountered.

Of interest, except for renal involvement, anti-CA II was also markedly associated with thrombocytopenia in our patients. As CA has been found in erythrocytes,[4] we infer that anti-CA antibody may bind CA and discrupt its function in other blood cells as well. But the mechanism involving blood cells still needs more researches. Other studies also regard anti-CA II as an anti-retinal antibody that penetrates the target cells and triggers retinal degeneration in retinopathies, and is associated with uveitis as well.[18,19] Given that CA II is a crucial enzyme in maintaining pH and proton pump activity in retinal cells, the inhibition of catalytic activity of CA II by the specific antibody is considered to cause decrease of intracellular pH and increase of intracellular calcium, ending up with retinal cell apoptosis and impact in retinal pathophysiology.[19,20]

In addition, in our study, as an autoantibody produced by B cells, anti-CA II antibody was related to hyperimmunogloblinemia, hypergammopathy, hypocomplementemia and higher disease activity score, which reflected the hyperactivity of abnormal

Table 4

Comparison of clinical and laboratory characteristics of pSS patients after stratification on dRTA.

Characteristics	dRTA (+) (n = 27)	dRTA (-) (n = 96)	*P* value
General information			
Female	27 (100)	92 (95.8)	.575
Age (yr)	53.0 (51.0–57.0)	56.0 (50.0–64.0)	.316
Disease duration (yr)	10.0 (12.0–16.0)	7.1 (4.0–12.9)	.157
Blood test parameters			
WCC (10^9/L)	4.2 (3.3–6.2)	4.4 (3.2–6.8)	.725
Hemoglobin (g/L)	116.0 (101.0–125.0)	117.5 (105.3–127.0)	.358
Platelet (10^9/L)	162.4 ± 75.1	171.4 ± 90.2	.603
Creatine (μmol/L)	94.0 (58.0–109.0)	55.5 (50.0–66.0)	<.001
Na (mmol/L)	142.3 ± 2.6	141.9 ± 2.9	.491
K (mmol/L)	3.41 ± 0.47	3.76 ± 0.40	<.001
Cl (mmol/L)	110.8 ± 4.0	107.2 ± 2.7	<.001
CO_2CP (mmol/L)	22.5 ± 4.0	24.3 ± 2.7	.034
Urine test parameters			
Fasting urine pH	6.67 ± 0.59	6.23 ± 0.50	<.001
Bicarbonate (mmol/L)	16.62 (13.19–22.10)	15.00 (10.20–21.30)	.144
TA (mmol/L)	3.40 (1.70–8.07)	8.14 (3.62–12.56)	<.001
NH_4^+ (mmol/L)	17.45 (15.22–28.61)	25.03 (20.07–36.38)	.002
RBP (μg/L)	0.57 (0.15–2.50)	0.26 (0.01–0.57)	.009
β2-MG (μg/L)	880.0 (534.0–5000.0)	303.5 (20.0–663.5)	<.001
NAG (U/L)	11.20 (0.01–17.10)	8.65 (2.84–12.90)	.572
24h urine protein (g/d)	0.45 (0.09–0.74)	0.08 (0.06–0.15)	<.001
Immunological indexes and disease activity score			
CRP (mg/L)	2.91 (1.79–8.76)	2.98 (1.82–6.09)	.714
ESR (mm/h)	27.0 (14.0–41.0)	29.0 (15.0–50.8)	.864
IgG (g/L)	17.7 (11.1–21.9)	17.3 (12.2–24.8)	.976
IgA (g/L)	3.46 (1.71–5.02)	3.20 (2.06–4.95)	.732
IgM (g/L)	1.09 (0.69–1.98)	1.07 (0.72–1.56)	.418
Complement 3 (g/L)	0.784 (0.591–0.943)	0.955 (0.768–1.070)	.008
Complement 4 (g/L)	0.179 ± 0.044	0.188 ± 0.080	.551
γ-globulin (%)	24.6 (17.4–27.4)	22.4 (18.3–29.0)	.830
RF (IU/ml)	44.3 (21.2–92.7)	27.9 (10.0–397.0)	.895
ANA	27 (100)	86 (89.6)	.177
Anti-SSA	21 (77.8)	75 (78.1)	.969
Anti-SSB	8 (29.6)	30 (31.3)	.872
Anti-α-fodrin (U/ml)	9.690 (3.365–15.770)	5.601 (2.547–11.726)	.054
Anti-CA II level (EU/ml)	0.960 (0.761–1.086)	0.877 (0.775–1.116)	.728
Anti-CA II positive	14 (51.9)	40 (41.7)	.346
ESSDAI score	5 (4–7)	4 (3–5.75)	.027

The data was expressed as mean ± S.D., or median (IQR), or n (%) as appropriate.

ANA = antinuclear antibody, CO_2CP = carbon dioxide combining power, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, dRTA = distal renal tubular acidosis, ESSDAI = the EULAR Sjögren Syndrome Disease Activity Index, IgA = immunoglobulin A, IgG = immunoglobulin G, IgM = immunoglobulin M, NAG = N-acetyl-β-D-glucosaminidase, pSS = primary Sjögren syndrome, RBP = retinol binding protein, RF = rheumatoid factor, TA = titratable acid, WCC = white cell count, β2-MG = β2-microglobulin.

B cells and systemic damage. Even though CA II was proven to induce autoimmune features in mice, so far, how anti-CA II is produced and the exact role of CA II antigen in the development of autoimmunity in pSS still remain unclear.

Admittedly, our study has limitations. In clinical practice, the evaluation of RTA and urinary acidification function are usually influenced by many factors, such as diet, respiratory problems and treatments. We have tried to minimize their impact by standardizing the sample collection. Maybe larger numbers and more controlled recruitment of patients could provide better outcome. Besides, as an exploratory study, we did not adjust multiple testing across many tests in the comparison of complications of pSS patients, leading to a weakness of this study. The correlation of anti-CA II and thrombocytopenia need to be tested in further confirmatory studies.

5. Conclusion

In conclusion, anti-CA II antibody particularlly reflected bicarbonic reabsoption dysfunction of proximal tubules, and was correlated with hematologic impairment and increased disease activity in pSS. It is promising that anti-CA II antibody could serve as a potential sensitive biomarker of systemic injury of pSS, even a target of treatment in the future. Further investigation is warranted to reveal the complicated role and mechanism of anti-CA II antibody in pSS.

Author contributions

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