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High levels of antibodies to citrullinated α-enolase peptide-1 (CEP-1) identify erosions and interstitial lung disease (ILD) in a Chinese rheumatoid arthritis cohort

Yudong Liu[a,*], Chenxi Liu[b], Liubing Li[b], Fengchun Zhang[b], Yongzhe Li[c,*], Shulan Zhang[b,*]

[a] Department of Clinical Laboratory, Peking University People's Hospital, Beijing, China
[b] Department of Rheumatology and Clinical Immunology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Key Laboratory of Rheumatology and Clinical Immunology, Ministry of Education, Beijing, China
[c] Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China

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ABSTRACT

We evaluated the clinical performance of anti-CEP-1 in a Chinese rheumatoid arthritis (RA) cohort. A total of 264 subjects were tested, including 101 RA patients, 38 juvenile idiopathic arthritis (JIA) patients, 46 disease control (DC) and 79 healthy controls (HC). The presence of anti-CEP-1 in patients with RA, JIA, DCs and HC were 61.4%, 13.2%, 15.2% and 5.1%, respectively. Anti-CCP2 demonstrated the highest positive likelihood ratio of 10.11 in the diagnosis of RA, followed by RF (8.88) and anti-CEP-1 (5.82). Anti-CEP-1 positive RA patients displayed significantly higher DAS28 compared to anti-CEP-1 negative RA patients ($p = .045$). Significant associations were identified between anti-CEP-1 and joint erosions at anti-CEP-1 value of > 124.78 U/ml ($p = .0026$) and between anti-CEP-1 and ILD at anti-CEP-1 value of > 185.91 U/ml ($p = .0222$). Our findings indicate that anti-CEP-1 may not be able to replace anti-CCP2 for routine diagnosis for RA, but they may be helpful for subtyping of the disease.

1. Introduction

Rheumatoid arthritis (RA) is a chronic and progressive autoimmune joint disorder characterized by synovial inflammation and joint destruction. RA also exhibited a heterogeneous clinical spectrum, characterized by various extra-articular manifestations (EAMs). Among those EAMs, cardiovascular disease and pulmonary involvement are the two most frequent causes of death in RA [1–3]. Although the autoantigens that trigger and sustain the immune response in RA remain unclear, the presence of autoantibodies against citrullinated peptides (ACPA) represents a hallmark feature of Rheumatoid arthritis (RA) [4–6]. ACPA are found in the sera of 60%–80% of patients with RA [7] and the presence of ACPA in serum is associated with a more severe disease course [8,9].

The citrullinated autoantigens targeted by those ACPA arise from a process called citrullination, a calcium-dependent posttranslational modification of peptidylarginine to peptidylcitrulline, which is mediated by peptidylarginine deiminases (PADs) enzymes [10]. Citrullinated proteins are not only present in inflamed synovium, they also can be found in extra-articular tissues, such as the lungs. Interestingly, it has been shown that smoking may induce citrullination in the lungs, resulting in the local accumulation of citrullinated proteins [11,12]. Those citrullinated proteins may be then presented to T cells in the context of specific shared epitope (SE)-containing HLA-DRB1 alleles, which may initiate a specific anti-citrulline immune response [13].

The presence of antibodies to citrullinated proteins, as detected by the anti-cyclic citrullinated peptide (anti-CCP) test, is now included as a criterion in the revised classification criteria for RA [14]. In addition to their diagnostic potential, those ACPAs may exert a pathogenic role through formation of immune complexes (IC) with citrullinated host cognate antigens in the joint and by activation of downstream inflammatory pathways via complement fixation and Fcγ receptor activation [15]. The current 'golden standard' for detection of ACPA is the second generation of anti-CCP (anti-CCP2) assay. Interestingly, the peptides in the CCP2 assay or the filaggrin peptides in the CCP1 assay are artificial peptides that are thought to be absent in the human joint. Thus, the CCP assay does not provide information regarding the underlying antigen specificities that initiate and/or perpetuate pathogenic responses in the joint.

* Corresponding authors.
E-mail addresses: yudongliu1983@126.com (Y. Liu), LiYZ@pumch.cn (Y. Li), shulanpumch@126.com (S. Zhang).

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α-enolase is one of the physiologic proteins that are targeted by ACPA in RA [16]. Autoantibodies to the immunodominant epitope in α-enolase, namely peptide 1 of citrullinated α-enolase (anti-CEP-1), are present in 37–62% of patients with RA [17–19]. In patients with RA, the levels of α-enolase are significantly elevated in synovial membrane as well as synovial fluid [20]. In addition, anti-CEP-1 antibodies are preferentially accumulated in rheumatoid synovial fluid compared to the serum, suggesting their involvement in local inflammatory response [21]. Importantly, Mahdi et al. reported that citrullinated α-enolase is a major citrullinated autoantigen that links smoking to genetic risk factors in the development of RA [22]. Thus, anti-CEP-1 antibodies, which target a true physiological protein, may represent a better marker than the artificial anti-CCP test in the diagnosis and evaluation disease severities in RA.

However, data regarding the ability of anti-CEP-1 in predicting the development of erosive disease as well as their potential associations with EAMs remains conflicting. A possible explanation for those discrepancies is the differences in genetic/environmental factors as well as smoking habits, as suggested by Wegner et al. [23]. In fact, a recent study demonstrated that although the total proportions of individuals positive for ACPAs are similar, serum ACPA fine specificities differ between Malaysian RA patients and Swedish RA populations, indicating variations in genetic and/or environmental factors may determine different patterns of ACPA fine specificities [24]. To our knowledge, few, if any, studies have assessed the clinical relevance of anti-CEP-1 in Chinese patients with RA. Since anti-CEP-1 target a true physiological protein, whose levels are significantly increased in the joint in RA, it is of paramount importance to evaluate the clinical utility of anti-CEP-1 in the diagnosis of RA in a Chinese RA cohort, particularly in their prognostic value for erosive disease and EAMs.

2. Materials and methods

2.1. Subjects and specimen collections

Sera from a total of 264 subjects were collected and analyzed, including 101 patients with RA, 38 patients with juvenile idiopathic arthritis (JIA), 46 patients with various diseases as disease control (DC), and 79 healthy controls. DC included 22 patients with Osteoarthritis (OA), 5 patients with Ankylosing spondylitis (AS), 5 patients with Adult onset Still's disease (AOSD), 4 patients with Psoriasis, 1 patient with Chronic recurrent multifocal osteomyelitis, 1 patient with Systemic lupus erythematosus (SLE), 1 patient with Polymyositis (PM), 1 patient with Dermatomyositis (DM), 1 patient with Reactive arthritis, 1 patient with systemic vasculitis (SV), 1 patient with gout, 1 patient with undifferentiated arthritis, 1 patient with lymphoma, and 1 patient with leukemia. RA was diagnosed according to 1987 American College of Rheumatology (ACR) criteria [25].

2.2. Data collection

Data on demographic features and clinical and laboratory findings were retrospectively collected. Clinical features included age at first complaint, age at diagnosis, disease duration, morning stiffness, swollen joint count (SJC), tender joint count (TJC), rheumatoid nodules, interstitial lung disease (ILD), joint erosions. Disease Activity Score (DAS28) was assessed from the number of SJC and TJC, erythrocyte sedimentation rate (ESR) and Visual Analogue Scale (VAS) for global health [26]. Study protocols were reviewed and approved by the Ethical Committee of Peking Union Medical College Hospital (PUMCH) and informed consents were obtained from all participants.

2.3. Serum autoantibodies and rheumatoid factor (RF) determination

Serum ACPA were determined by anti-CCP2 IgG ELISA (Euro Diagnostica, Malmö, Sweden) according to the manufacturer's instructions. The cutoff values for positivity were set as ≥25 U/ml based on the recommendations by the manufacturer. Serum RF were determined by the nephelometry method (Behring, Germany). The cutoff values for positivity were set as ≥20 U/ml based on the recommendations by the manufacturer. Serum IgG anti-CEP-1 were determined by an anti-CEP-1 ELISA (IgG) kit (Euroimmun, Germany) according to manufacturer's instructions, as described by other studies [27]. Briefly, the serum was diluted at 1:101 ratio and the samples were considered positive if the antibody titer was ≥20 relative units/ml. The intra- and inter-assay coefficients of variation (CV) were < 10% for all the tests, which was in accordance with the Clinical and Laboratory Standards Institute (CLSI) protocol EP15-A2 [28]. Specifically, the intra- and inter-assay CV of anti-CCP2 IgG ELISA were 2.1%–3.5% and 4.1%–7.8%, respectively, while the intra- and inter-assay CV of anti-CEP-1 IgG ELISA were 1.8%–4.2% and 3.7%–8.2%, respectively.

2.4. Statistical analysis

Continuous variables were compared using the Mann-Whitney *U* test. The Kruskal-Wallis (KW) test was utilized to calculate the differences between groups, followed by Dunnett's T2 test. The $\chi 2$ test or Fisher exact test was utilized for comparison of categorical variables. Correlations between anti-CCP2 or anti-CEP-1 and RA-associated clinical manifestations were determined by logistic regression models. Spearman's correlation test was performed to analyze the correlations between anti-CEP-1 and anti-CCP2 antibodies, between anti-CEP1 and RF or between anti-CEP-1 and DAS28. p values of < 0.05 were considered statistically significant. All statistical analyses were performed by SPSS 20.0 statistical software package (SPSS Inc., Chicago, Illinois, USA) and MedCalc (MedCalc Software, Ostend, Belgium).

3. Results

3.1. Levels of anti-CEP-1 were significantly elevated in patients with RA

Overall, the levels of anti-CEP-1 were significantly increased in patients with RA compared with patients with JIA and other DCs as well as HC ($p < .05$) (Fig. 1A). No significant differences in the levels of anti-CEP-1 were observed between patients with JIA and other DCs (Fig. 1A). The presence of anti-CEP-1 in patients with RA, JIA, DCs and HC were 61.4%, 13.2%, 15.2% and 5.1%, respectively (Table 1). The presence of anti-CCP2 and RF in patients with RA, JIA, DCs and HC were 76.2% and 77.2%, 13.2% and 10.0%, 8.7% and 9.3%, 1.3% and 6.3%, respectively (Table 1).

3.2. Relationships between anti-CEP-1 and anti-CCP2 or RF in patients with RA

Significantly elevated levels of anti-CEP-1 were found in anti-CCP positive RA patients compared to anti-CCP negative RA patients (Fig. 1B) or in RF positive RA patients compared to RF negative RA patients (Fig. 1C). Consistently, significant correlations between anti-CEP-1 and anti-CCP2 ($R^2 = 0.609$, $p < .0001$) (Fig. 2A) or between anti-CEP-1 and RF ($R^2 = 0.497$, $p < .0001$) were observed (Fig. 2B). Venn diagram was utilized to further assess the distributions and relationships among anti-CEP-1, anti-CCP2 and RF (Fig. 3). A total of 54 RA patients (53.5%) were positive for all of those autoantibodies, while 17 RA patients (16.8%) were positive for both anti-CCP2 and RF. Of interest, 5 of anti-CCP2 negative RA patients (20.8%) were positive for anti-CEP-1 (Fig. 3).

3.3. The predictive power of anti-CEP-1, anti-CCP2 and RF in the diagnosis of patients with RA vs. controls

Assay performance parameters of anti-CEP-1, anti-CCP2 and RF were calculated (Table 2). Anti-CCP2 demonstrated the highest positive

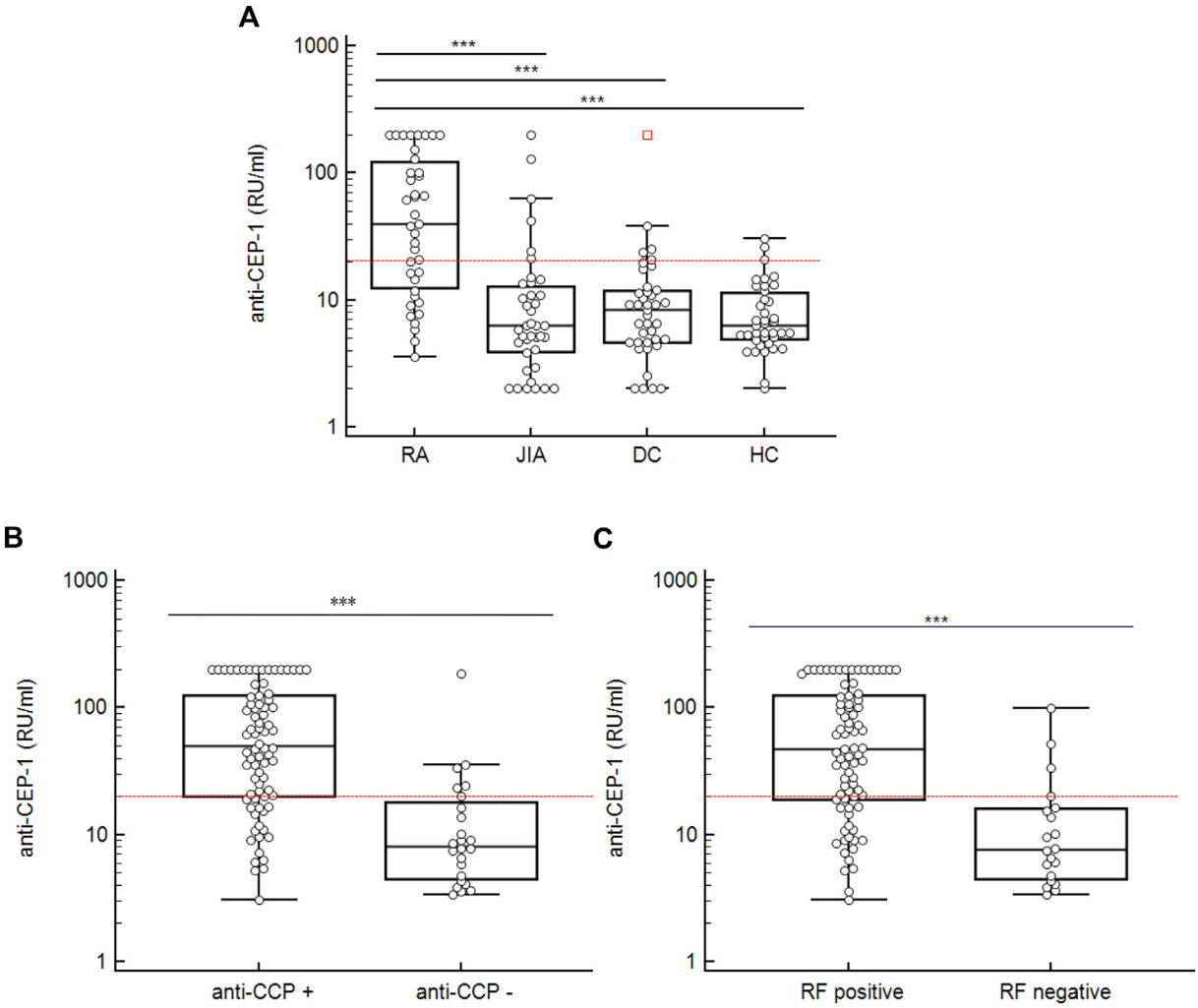

Fig. 1. (**A**). Levels of anti-CEP-1 in patients with rheumatoid arthritis (RA), patients with juvenile idiopathic arthritis (JIA), Disease controls (DC) and Healthy controls (HC); Statistical analysis was performed by the Kruskal-Wallis (KW) test, followed by Dunnett's T2 test. RA, $n = 101$; JIA, $n = 38$; DC, $n = 46$; HC, $n = 79$ (**B**). Levels of anti-CEP-1 between anti-CCP2+ RA patients and anti-CCP2- RA patients; Statistical analysis was performed by the Mann-Whitney U test. Anti-CEP-1$^+$, n = 62; anti-CEP-1$^-$, n = 39 (**C**). Levels of anti-CEP-1 between rheumatoid factor (RF)$^+$ RA patients and RF$^-$ RA patients; Statistical analysis was performed by the Mann-Whitney *U* test. Anti-CEP-1$^+$, $n = 62$; anti-CEP-1$^-$, $n = 39$. anti-CCP2, anti-cyclic citrullinated peptide test; anti-CEP-1, anti-peptide 1 of citrullinated α-enolase.

Table 1
Demographic, clinical characteristic, and autoantibody profiles between patients with RA and controls.

	RA	JIA	DC	HC	*p* Value
Number, *n*	101	38	46	79	
Female gender, *n* (%)	75/101 (74.3)	24/38 (63.2)	27/46 (58.7)	51/28 (64.6)	0.278
Age, median (quartiles), years	54 (45, 62)	11 (6.25, 14)	57 (36, 64)	50 (40, 57)	< 0.0001*
Age at first complaint, median(quartiles), years	44 (31, 52)	4.5 (3, 7)	44 (23.75, 52.75)	N/A	< 0.0001*
Age at diagnosis, median (quartiles), years	45 (33, 54)	5.5 (3.625, 8)	49 (30.5, 58.75)	N/A	< 0.0001*
Duration, median (quartiles), years	7 (2, 16)	3 (1.125, 7)	6 (1.5, 14.75)	N/A	< 0.0001*
DAS 28, mean (SD)	3.99 (1.85)	N/A	N/A	N/A	N/A
Rheumatoid nodules, n (%)	15 (14.9)	N/A	N/A	N/A	N/A
Erosions, n (%)	37 (36.6)	N/A	N/A	N/A	N/A
Interstitial lung disease (ILD), n (%)	23 (22.8)	N/A	N/A	N/A	N/A
Morning stiffness, n (%)	59 (58.4)	N/A	N/A	N/A	N/A
Anti-CCP2, n (%)	77/101, (76.2)	5/38, (13.2)	4/46, (8.7)	1/79, (1.3)	< 0.0001**
Rheumatoid factor (RF), n (%)	78/101, (77.2)	2/20, (10.0)	4/43, (9.3)	5/79, (6.3)	< 0.0001**
Anti-CEP-1, n (%)	62/101, (61.4)	5/38, (13.2)	7/46, (15.2)	4/79, (5.1)	< 0.0001**

The Kruskal-Wallis (KW) test was used to calculate the differences among all the groups, followed by Dunnett's T2 test. * *p*-Value indicates significant differences between JIA and other groups. ** *p*-Value indicates significant differences between RA and other groups. RA, Rheumatoid arthritis; juvenile idiopathic arthritis (JIA); disease control (DC); healthy controls, DAS 28, Disease Activity Score 28; anti-CCP2, anti-cyclic citrullinated peptide test; anti-CEP-1, anti-peptide 1 of citrullinated α-enolase; N/A, not applicable.

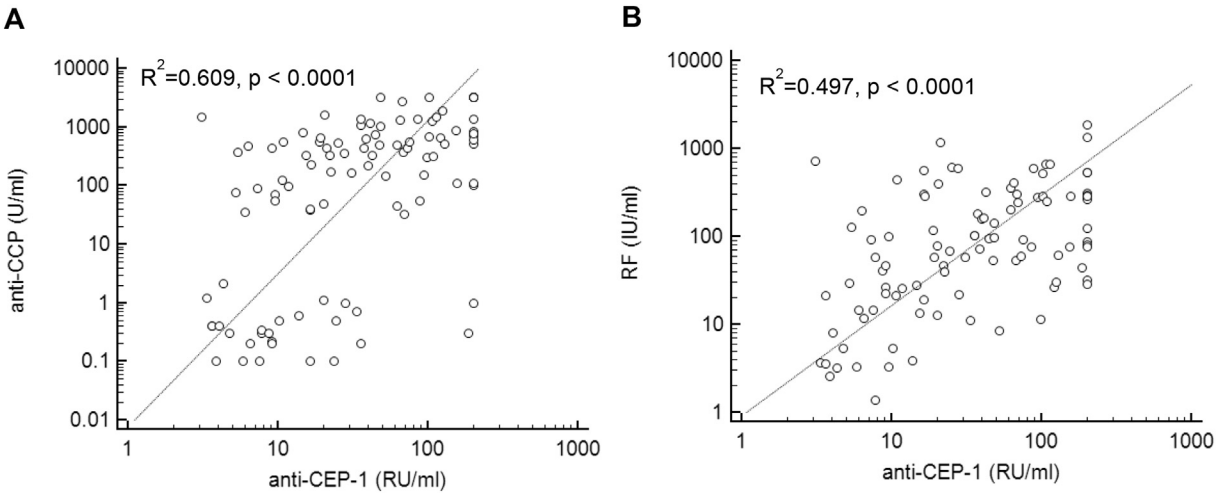

Fig. 2. Correlations between anti-CEP-1 and anti-CCP2 antibodies (**A**), between anti-CEP1 and rheumatoid factor (RF) (**B**) or between anti-CEP-1 in patients with rheumatoid arthritis (RA). *N* = 101. anti-CCP2, anti-cyclic citrullinated peptide test; anti-CEP-1, anti-peptide 1 of citrullinated α-enolase.

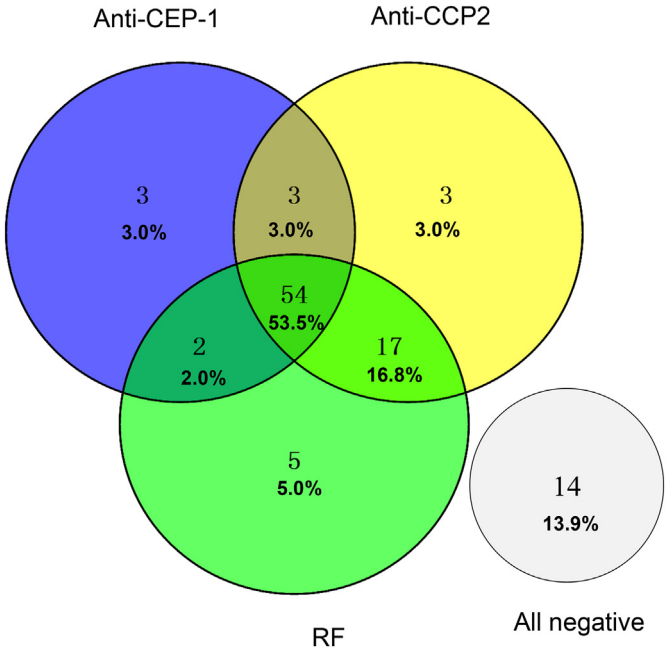

Fig. 3. Relationships among anti-CEP-1, anti-CCP2 and rheumatoid factor (RF) in patients with rheumatoid arthritis (RA). N = 101. anti-CCP2, anti-cyclic citrullinated peptide test; anti-CEP-1, anti-peptide 1 of citrullinated α-enolase.

likelihood ratio (LR+) of 10.11 with a sensitivity of 76.24% and a specificity of 92.46%, followed by RF (LR+ of 8.88, sensitivity of 77.23% and specificity of 91.3%) and anti-CEP-1 (LR+ of 5.82, sensitivity of 61.39% and specificity of 89.45%) (Table 2). The clinical performance of combination of anti-CEP-1 and/or anti-CCP2 were also evaluated. Of note, either anti-CEP-1$^+$ or anti-CCP2$^+$ displayed a sensitivity of 81.19% with a LR+ of 5.57, while both anti-CEP-1$^+$ and anti-CCP2$^+$ demonstrated a LR+ of 16.04 with a diagnostic sensitivity of 56.44% (Table 2).

3.4. Demographic features and clinical characteristics between anti-CEP-1 positive RA patients and anti-CEP-1 negative RA patients

Demographic features and clinical characteristics were compared between anti-CEP-1 positive RA patients and anti-CEP-1 negative RA patients (Table 3). Importantly, anti-CEP-1 positive RA patients displayed significantly higher levels of DAS28 compared to anti-CEP-1 negative RA patients (p = .045). In addition, the presence of erosions was higher in anti-CEP-1 positive RA patients compared to anti-CEP-1 negative RA patients (41.9% vs. 28.2%). Interestingly, anti-CEP-1 positive RA patients tended to be older at the time of first complaint (p = .092) or at the time of diagnosis (p = .065). Smoking has been considered to contribute to the citrullination of autoantigens in RA [11]. Of note, 25.8% of anti-CEP-1 positive RA patients were smokers, which was higher than that in anti-CEP-1 negative RA patients (25.8% vs. 15.4%), although no significant differences were identified.

Table 2
The predictive power of multiple autoantibodies in the diagnosis of patients with RA vs. Disease Controls.

	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)	LR+ (95% CI)	LR − (95% CI)
anti-CCP2	76.24 (66.54–83.90)	92.46 (87.64–95.57)	83.70 (74.21–90.29)	88.46 (83.13–92.32)	10.11 (6.14–16.65)	0.26 (0.18–0.36)
RF	77.23 (67.61–84.74)	91.30 (85.57–94.99)	84.78 (74.44–91.13)	86.47 (80.18–91.05)	8.88 (5.32–14.81)	0.25 (0.17–0.36)
anti-CEP-1	61.39 (51.14–70.75)	89.45 (84.12–93.20)	74.70 (63.75–83.32)	82.03 (63.75–83.32)	5.82 (3.77–8.97)	0.43 (0.34–0.55)
Either anti-CEP-1+ or anti-CCP2+	81.19 (71.93–88.02)	85.43 (79.57–89.87)	73.87 (64.52–81.54)	89.95 (84.53–93.68)	5.57 (3.93–7.90)	0.22 (0.15–0.33)
Both anti-CEP-1+ and anti-CCP2+	56.44 (46.22–66.15)	96.48 (92.59–98.45)	89.06 (78.16–95.12)	81.36 (75.67–86.00)	16.04 (7.60–33.88)	0.45 (0.36–0.56)

RF, Rheumatoid factor; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR-, negative likelihood ratio. In brackets, 95% CI, 95% confidence intervals (CI); anti-cyclic citrullinated peptide test; anti-CEP-1, anti-peptide 1 of citrullinated α-enolase.

Table 3
Demographic, clinical characteristic and autoantibody profiles between anti-CEP-1 negative and anti-CEP-1-positive RA patients.

	anti-CEP-1$^-$	anti-CEP-1$^+$	*p* Value
Number, *n*	39	62	
Female gender, *n* (%)	30 (76.9)	45 (72.6)	0.627
Age, median (quartiles), years	53 (39.5, 62)	56 (48.25, 62.75)	0.150
Age at first complaint, median (quartiles), years	40 (25, 52)	45.5 (35, 52)	0.092
Age at diagnosis, median (quartiles), years	40 (26, 52)	48.5 (35.5, 54)	0.065
During, median(quartiles), years	8 (2.75, 15)	5.5 (2, 16)	0.617
Smoking, n (%)	6 (15.4)	16 (25.8)	0.217
Morning stiffness, n (%)	21 (53.8)	38 (61.3)	0.347
Swollen joint count, median (quartiles)	0 (0, 3)	1 (0, 6.75)	0.247
Tender joint count, median (quartiles)	1 (0, 6.5)	2 (0, 7.75)	0.316
DAS 28, mean (SD)	3.53 (1.52)	4.29 (1.98)	0.045
Erosions, n (%)	11 (28.2)	26 (41.9)	0.163
Interstitial lung disease (ILD), n (%)	9 (23.1)	14 (22.6)	0.954
Rheumatoid nodules, n (%)	6 (15.4)	9 (14.5)	0.889
anti-CCP2, n (%)	20 (51.3)	57 (91.9)	< 0.001
Rheumatoid factor (RF), n (%)	22 (56.4)	56 (90.3)	< 0.001

DAS 28, Disease Activity Score 28; anti-cyclic citrullinated peptide test; anti-CEP-1, anti-peptide 1 of citrullinated α-enolase.

3.5. Associations between anti-CEP-1 and RA-associated clinical manifestations

No significant associations were found between anti-CEP-1 and RA-associated clinical manifestations, including joint erosions or interstitial lung disease (ILD) using the cutoff values recommended by the manufacturer. However, after we recalculated the cut-off value using Youden's index, significant associations were identified between anti-CEP-1 and joint erosions at anti-CEP-1 value of > 124.78 U/ml (OR, 5.24; $p = .0026$) and between anti-CEP-1 and ILD at anti-CEP-1 value of > 185.91 U/ml (OR, 3.83; $p = .0222$), while no significant associations were observed between anti-CCP2 and joint erosions or ILD, no matter how cut-off values were set up (Table 4). Those findings indicate high levels of anti-CEP-1 could contribute to identify RA patients with joint erosions or ILD.

4. Discussion

In the present study, we evaluated the clinical performance of anti-CEP-1 in the diagnosis of RA in a well-defined Chinese cohort. Although anti-CEP-1 did not exhibit better diagnostic potentials than the current routinely used markers (anti-CCP2 and RF), high levels of anti-CEP-1, but not anti-CCP2, did demonstrate promising potentials in identifying RA patients with erosions or ILD. In addition, the combination of anti-CCP2 with anti-CEP-1 data increased the overall sensitivity of ACPAs in our cohort to 81.2%. Collectively, our findings delineate the clinical relevance of anti-CEP-1 in the diagnosis of RA, indicating that this marker may be helpful in identifying clinical subsets in patients with RA.

Table 4
Diagnostic power of anti-CCP2 and anti-CEP-1 autoantibodies in predicting RA-associated clinical manifestations.

RA-associated clinical manifestations	OR	95% CI	*p* Value
Erosions			
anti-CEP-1 > 20 U/ml	1.84	0.78, 4.35	0.166
anti-CEP-1 > 124.78 U/ml[a]	5.24	1.78, 15.39	0.0026
anti-CCP > 25 U/ml	1.55	0.57, 4.18	0.3866
anti-CCP > 97 U/ml	2.27	0.92, 5.58	0.0742
RA-associated Interstitial lung disease (ILD)			
anti-CEP-1 > 20 U/ml	2.41	0.82, 7.09	0.1112
anti-CEP-1 > 185.91 U/ml[a]	3.83	1.21, 12.10	0.0222
anti-CCP > 25 U/ml	0.64	0.23, 1.80	0.3945
anti-CCP < 760 U/ml	1.09	0.10, 0.67	0.0747

[a] The values were calculated based on Youden's index as a way of summarizing the performance of a diagnostic test. OR, odds ratio; 95% CI, 95% confidence intervals (CI); anti-CCP2, anti-cyclic citrullinated peptide test; anti-CEP-1, anti-peptide 1 of citrullinated α-enolase.

One of the most important findings from our study is the significant associations between high levels of anti-CEP-1 with erosions and ILD. Rather than anti-CCP2, which target artificial citrullinated peptides, anti-CEP-1 targets a true physiological protein, whose levels are significantly increased in the synovial membrane and synovial fluid in RA [16,20]. Of interest, it has been shown that the autoantibodies against citrullinated form of physiological antigens also cross-react with other noncitrullinated epitopes on the naïve proteins, directly causing proteoglycan depletion of cartilage and severe arthritis in mice [5]. In addition, IC complexes containing citrullinated proteins can serve as damage-associated molecular patterns (DAMPs) to activate macrophage for pro-inflammatory cytokine production and propagate neutrophil activation [29]. Thus, our findings of significant correlations between high levels of anti-CEP-1 and RA-associated clinical manifestations are consistent with those findings, supporting that anti-CEP-1 may play a pathogenic role in RA-associated clinical manifestations. Of interest, we failed to identify such significant associations when anti-CEP-1 were at low levels. It is possible that ACPA are only pathogenic when they reach high levels.

The mechanism explaining why high levels of anti-CCP2 did not exhibit significant associations with erosions or ILD is unclear. Of note, anti-CEP-1 and anti-CCP2 displayed significant correlations ($R^2 = 0.609$). As anti-CEP-1 target physiological antigens while anti-CCP2 target artificially generated synthetic peptides with no homology to known proteins, it is possible that the significant correlations between ACPAs and RA-associated clinical manifestations were determined the fine specificity of each ACPA and citrullinated peptides from the real physiological antigens.

In this study, the LR+ value of anti-CEP-1 (LR+ of 5.82) was lower than those from anti-CCP2 (LR+ of 10.11) or RF (LR+ of 8.88). Thus, our findings showed that the diagnostic potential of anti-CEP-1 was not superior to the conventional markers for RA diagnosis, and should not be included in the routine laboratory diagnostic process. However, it is worth noting that 5 of anti-CCP2 negative RA patients (20.8%) were positive for anti-CEP-1, thus the addition of anti-CEP-1 to anti-CCP2 (either anti-CEP-1$^+$ or anti-CCP2$^+$) increased the overall diagnostic sensitivity of ACPAs to 81.19% with a LR+ of 5.57, while both anti-CEP-1$^+$ and anti-CCP2$^+$ increased the LR+ to 16.04, although the overall diagnostic sensitivity decreased to 56.44%. Interestingly, Lundberg et al. also reported that seven (23%) of the anti-CCP antibody-negative patients with RA had positive results on the anti-CEP-1 ELISA, and combining the anti-CCP with the anti-CEP-1 ELISA results increased the overall sensitivity of ACPAs in this cohort to 78% [17], which is similar to our data.

Several studies also suggested ACPAs may play a role in JIA [30,31]. Gilliam et al. reported that 32% JIA patients demonstrated reactivity to citrullinated fibrinogen, 24% of JIA patients were positive to anti-citrullinated type II collagen, while 10.5% JIA patients positive for anti-citrullinated vimentin and 9% of JIA patients positive for anti-CEP-1 [30,31]. Those findings have indicated that the presence of autoantibodies to citrullinated antigens in JIA patients is highly diverse, with anti-citrullinated fibrinogen and anti-citrullinated type II collagen as the predominant ones. Interestingly, we included JIA as disease control to evaluate the diagnostic value of anti-CEP-1 in RA. We found that anti-CEP-1 were present in 13.2% JIA patients, which was similar to that from other studies [30]. While it remains unclear whether autoantibodies to each citrullinated proteins are differentially expressed

in JIA, the low prevalence of anti-CEP-1 in JIA and high prevalence of anti-CEP-1 in RA enable their diagnostic capacity to differentiate RA from JIA.

Our study has a number of notable strengths. To the best of our knowledge, our study represents the first study in China investigating the clinical performance of anti-CEP-1 in the diagnosis of RA. As variations in genetic and/or environmental factors influence the patterns of ACPA fine specificities [24], our findings will enable our understanding of the clinical utility of anti-CEP-1 in Chinese RA patients. It should be noted, however, that our study has several limitations. First, the sample size of this study was small, which may lead to potential analytical bias. Further studies with large cohorts are needed. Second, the subjects in our study were from a single institution, and these subjects were homogenous Han Chinese ethnic group. A multicenter study with various ethnic groups is needed to evaluate the generalizability of our results.

5. Conclusion

Our findings indicate that anti-CEP-1 may not be able to replace anti-CCP2 for routine laboratory diagnostic process for RA, but they may be helpful for subtyping of the disease.

Competing interests

The authors have no conflicting interests.

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Contributors

YLiu, FZ, YL and SZ designed the study. YLiu, CL, LL, FZ and SZ performed the experiments and analyzed the data. YLiu, YLi and SZ wrote the manuscript.

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