REVIEW ARTICLE

Diagnostic value of anti-citrullinated α-enolase peptide 1 antibody in patients with rheumatoid arthritis: A systematic review and meta-analysis

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Abstract

Aim: To evaluate the diagnostic value of anti-citrullinated α-enolase peptide 1 (anti-CEP 1) antibody in patients with rheumatoid arthritis (RA) by conducting a systematic review and meta-analysis.

Methods: The PubMed, Web of Science, Embase, Scopus, and Cochrane Library databases were searched for relevant studies published until September 23, 2020. A bivariate mixed-effects model was used to calculate the diagnostic indices from primary data of eligible studies. We performed meta-regression and subgroup analysis to explore the sources of heterogeneity.

Results: Twenty-four articles, with a total of 17 380 patients with RA and 7505 control participants, met the criteria for inclusion in the meta-analysis. The pooled sensitivity, specificity, and positive and negative likelihood ratios for the anti-CEP 1 antibody were 44% (95% CI: 38%-51%), 97% (95% CI: 96%-98%), and 14.81 (95% CI: 10.66-20.57) and 0.57 (95% CI: 0.52-0.64), respectively. The pooled positive and negative predictive values were 0.96 (95% CI: 0.95-0.97) and 0.53 (95% CI: 0.43-0.63), respectively. The area under the summary receiver operating characteristic curve was 0.86. Meta-regression indicated that the anti-CEP 1 antibody detection method may be a source of heterogeneity. The subgroup analysis of the group in which the anti-CEP 1 antibody was detected by using a commercial enzyme-linked immunosorbent assay (ELISA) kit had a sensitivity of 59% (95% CI: 50%-68%) and a specificity of 93% (95% CI: 85%-97%).

Conclusions: The anti-CEP 1 antibody had moderate RA diagnostic value with relatively low sensitivity and high specificity. An ELISA may increase the RA diagnostic sensitivity.

KEYWORDS

anti-citrullinated protein antibodies, anti-citrullinated α-enolase peptide 1 antibody, autoantibody, diagnosis, meta-analysis, rheumatoid arthritis

1 | INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disorder characterized by irreversible joint erosion, articular cartilage destruction, and synovial inflammation.[1] Additionally, patients with RA may have coexisting extra-articular manifestations, such as cardiovascular events, lung disease, and neurological involvement,[2,3] which could seriously affect the quality of life in RA patients. However, early diagnosis of RA and intervention can help achieve remission and reduce the possibility of RA-related disabilities.[4]

Autoantibodies are the hallmark of RA, of which anti-cyclic citrullinated peptide (anti-CCP) antibody and rheumatoid factor (RF) are routinely used to diagnose RA.[5] They are also recommended by the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) the criteria of which are used to diagnose RA. Nevertheless, using only anti-CCP antibody and RF is insufficient to identify some potential patients with early stage RA who are negative for anti-CCP antibody and RF.[6] Therefore, more novel autoantibodies are needed to allow identification of seronegative RA patients. Anti-citrullinated protein antibodies (ACPAs) have an important role in diagnosing RA. ACPAs interact with different citrullinated proteins as target antigens, including fibrinogen, type II collagen, vimentin, and α-enolase.[7] In particular, α-enolase, one of the key enzymes for glycolysis, is involved in the pathogenesis of RA.[8] In 2005, Kinloch et al.[9] first reported that citrullinated α-enolase peptide (CEP) was specific for RA, and they observed that CEP can be detected in the synovial fluid of patients with RA and that the anti-CEP 1 antibody had a higher level in synovial fluid than in serum.[10] These findings suggest that the anti-CEP 1 antibody may be produced from joint tissue, and it may better reflect the pathological changes involved in RA than the anti-CCP antibody that targets synthetic antigen but not physiological proteins. Additionally, anti-CEP 1 antibody can be detected in patients with seronegative RA,[11,12] suggesting that it helps with early diagnosis of RA.

Several studies have investigated the diagnostic value of anti-CEP 1 antibody for RA. However, the results from different studies have been inconsistent, and no published systematic review or meta-analysis has evaluated the diagnostic value of anti-CEP 1 antibody for RA. Therefore, we conducted this systematic review and meta-analysis to assess the RA diagnostic performance of the anti-CEP 1 antibody and identify factors that may affect its performance.

2 | METHODS

This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[13] The PRISMA checklist is shown in the supplementary files (see Appendix S1).

2.1 | Search strategy

The following 5 electronic databases were searched to retrieve relevant studies: PubMed, Web of Science, Embase, Scopus, and Cochrane Library. All studies were published prior to September 23, 2020, and we applied no language restriction. To construct the search strategy, the index terms were used as follows: autoantibody to citrullinated alpha enolase peptides 1, autoantibody to citrullinated α-enolase peptide 1, autoantibody to CEP-1, anti-CEP-1 antibody, rheumatoid arthritis, and RA. The detailed search strategy is presented in the supplementary files (see Appendix S2B).

2.2 | Study inclusion and exclusion criteria

Two investigators independently screened all the articles searched in the electronic databases. We included studies fulfilling the following inclusion criteria: (a) the diagnostic accuracy of anti-CEP 1 antibodies in RA was evaluated; (b) necessary data including sensitivity, specificity, false positives, and false negatives could be obtained or calculated from the study; (c) healthy donors or non-RA disease patients were enrolled in the study; (d) the diagnosis of patients with RA was based on the ACR or EULAR diagnostic criteria.

The following exclusion criteria were adopted: (a) studies without enough data to construct 2 × 2 contingency tables; (b) studies examining the diagnostic accuracy of the anti-CEP 1 antibody for future RA; (c) patient numbers with RA < 50; (d) tested samples were not in serum or plasma. (e) the study included duplicate data; (f) animal experiments.

2.3 | Data extraction

The process of data extraction was independently conducted by 2 investigators, and we extracted the essential information presented in the eligible articles, including the first author, published year, age, country where the study was performed, type of article, study design, method, plate and antibody used in enzyme-linked immunosorbent assay (ELISA), CEP-1 peptide sequence, diagnostic standard for RA, age, RA number, non-RA number, a cut-off of the method, diagnostic index, and anti-CEP 1 positive rate in patients with RA who were anti-CCP negative or positive. Any disagreements were resolved by reaching a consensus.

2.4 | Quality assessment

According to the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool,[14] 2 investigators independently assessed the quality of all eligible literature. QUADAS-2 evaluates 2 sections: risk of bias and concerns regarding applicability. Evaluation of the risk of bias comprised patient selection, index
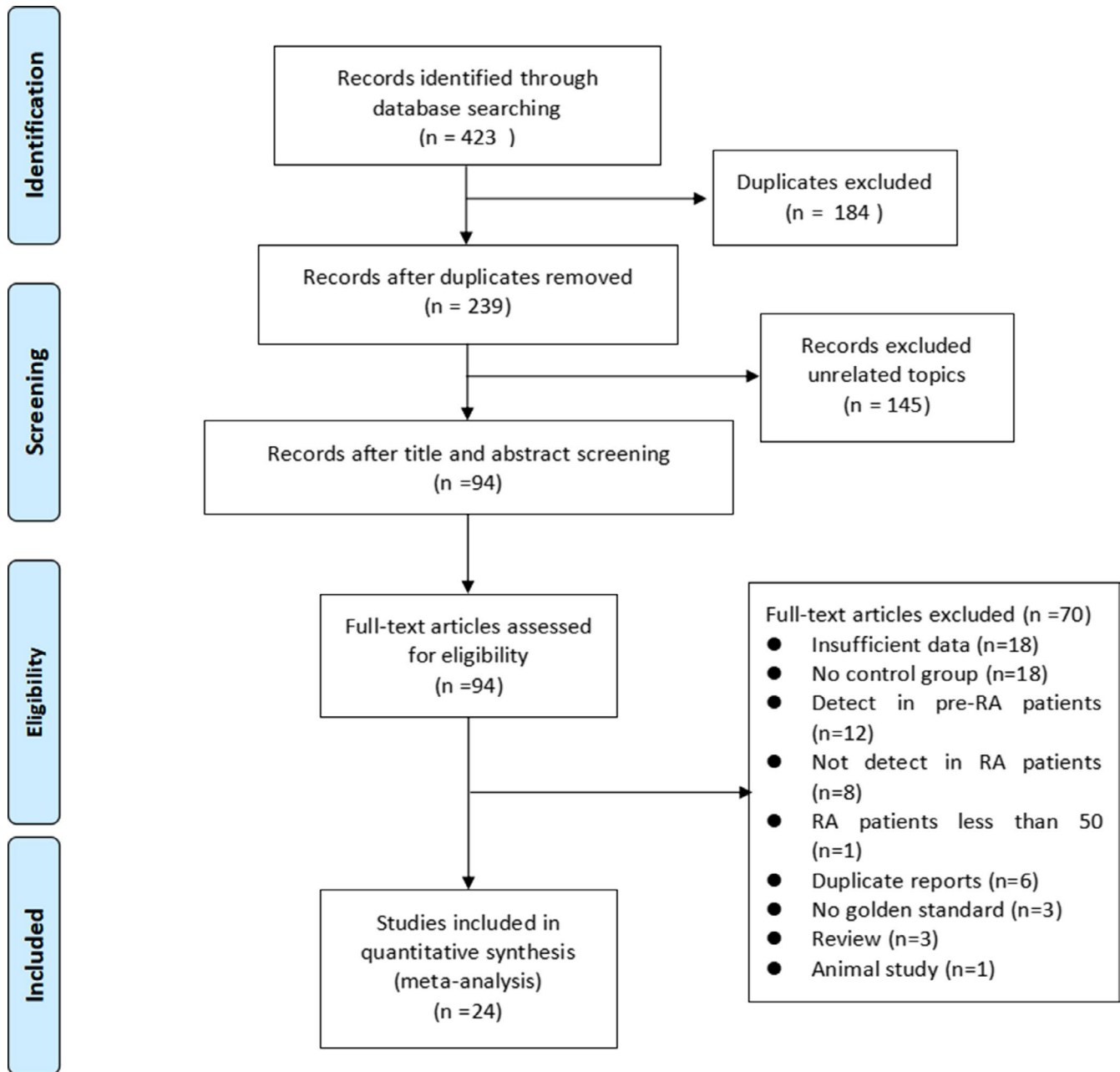
FIGURE 1 Flow diagram for screening studies and results

test, reference standard, flow and timing. The evaluation of the concerns regarding applicability included patient selection, index test, and reference standards. The risk was scored as high, low, or unclear according to the evaluating results of each section. Two investigators performed the quality assessment, and when there were inconsistent evaluation results, we resolved the disagreement through discussion.

2.5 | Statistical analysis

We used STATA 15 (Stata Corp, College Station, TX, USA), Meta-DiSc V.1.4 (Unit of Clinical Biostatistics team of the Ramony Cajal Hospital), and RevMan 5.3 (the Nordic Cochrane Center) software to perform the meta-analysis. A bivariate mixed-effects model was used to calculate the pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), positive predictive value (PPV), negative predictive value (NPV), and diagnostic odds ratio (DOR). We established a summary receiver operator characteristic (SROC) curve and calculated the area under the SROC curve (AUC) to evaluate the overall performance of the anti-CEP 1 antibody in patients with RA. The I^2 statistical test, which is an index for assessing heterogeneity, was used to detect heterogeneity within studies; a value of I^2 >50% indicates substantial heterogeneity. We tested for threshold effects, which could lead to heterogeneity of results due to inconsistent cut-off values applied in various studies,

TABLE 1 Some characteristics of 24 eligible studies in this meta-analysis

Author	Published y	Country	Type of article	Study design	Method	Plate and antibody brands of ELISA/ Microarray brand	CEP-1 peptide sequence	Diagnostic standard for RA
Zhou et al[15]	2019	China	Journal article	Case-control	ELISA	Commercial ELISA kit: Euroimmun	NR	2010 ACR/EULAR criteria
Ponikowska et al[16]	2019	Poland	Journal article	Case-control	ELISA	Commercial ELISA kit: Euroimmun	NR	2010 ACR/EULAR criteria
Liu et al[17]	2019	China	Journal article	Case-control	ELISA	Commercial ELISA kit: Euroimmun	NR	1987 ACR criteria
Rönnelid et al[18]	2018	Sweden	Journal article	Case-control	Microarray	Phadia AB	CKIHA(cit)EIFDS(cit) GNPTVEC (cyclic)	1987 ACR criteria
Meyer et al[19]	2018	South Africa	Journal article	Case-control	ELISA	Commercial ELISA kit: Euroimmun	NR	1987 ACR criteria
Jonsson et al[20]	2018	Norway	Journal article	Cross-sectional	Microarray	Phadia AB	CKIHA(cit)EIFDS(cit) GNPTVEC (cyclic)	2010 ACR/EULAR criteria
Alunno et al[21]	2018	Italy	Journal article	Case-control	ELISA	Commercial ELISA kit: Euroimmun	NR	1987 ACR criteria
Alunno et al[22]	2018	Italy	Journal article	Cross-sectional	ELISA	Commercial ELISA kit: Euroimmun	NR	2010 ACR/EULAR criteria
Too et al[23]	2017	Malaysia/ Sweden	Journal article	Case-control	Microarray	Phadia AB	NR	1987 ACR criteria
Schwenzer et al[24]	2017	United states	Journal article	Cross-sectional	ELISA	Plate: NR Antibody: Stratech	KIHA(cit)EIFDS(cit)GNPTVE	1987 ACR criteria
Cabrera-Villalba et al[25]	2017	Spain	Journal article	Cross-sectional	ELISA	Plate: NR Antibody: Jackson Immunoresearch	CKIHA(cit)EIFDS(cit) GNPTVEC (cyclic)	1987 ACR criteria
Brink et al[26]	2017	Sweden	Meeting abstract	Prospective study	Microarray	Phadia AB	NR	1987 ACR criteria
Reed et al[27]	2016	Sweden	Journal article	Cross-sectional	Microarray	Phadia AB	CKIHA(cit)EIFDS(cit) GNPTVEC (cyclic)	1987 ACR criteria
Choi et al[28]	2016	Korea	Journal article	Cross-sectional	ELISA	Plate: MaxiSorp antibody: Millipore	KIHA(cit)EIFDS(cit)GNPTVE	1987 ACR criteria
Quirke et al[29]	2015	United Kingdom	Journal article	Cross-sectional	ELISA	Plate: NR Antibody: Hybridoma Reagent Laboratory	KIHA(cit)EIFDS(cit)GNPTVE	2010 ACR/EULAR criteria
Kokkonen et al[30]	2015	Sweden	Journal article	Case-control	Microarray	Phadia AB	CKIHA(cit)EIFDS(cit) GNPTVEC (cyclic)	1987 ACR criteria
Umeda et al[31]	2013	Japan	Journal article	Case-control	ELISA	Plate: MaxiSorp Antibody: American Qualex International	CKIHA(cit)EIFDS(cit) GNPTVEC (cyclic)	1987 ACR criteria
Goules et al[32]	2013	Greece	Journal article	Case-control	ELISA	Plate: Costar Antibody: Jackson Immunoresearch	KIHA(cit)EIFDS(cit)GNPTVE	1987 ACR criteria

(Continues)

TABLE 1 (Continued)

Author	Published y	Country	Type of article	Study design	Method	Plate and antibody brands of ELISA/ Microarray brand	CEP-1 peptide sequence	Diagnostic standard for RA
Montes et al[33]	2012	Spain	Journal article	Case-control	ELISA	Plate: Pierce Antibody: NR	CKIHA(cit)EIFDS(cit)GNPTVEC (cyclic)	1987 ACR criteria
Hansson et al[34]	2012	Sweden	Journal article	Case-control	Microarray	Phadia Multiplexing Diagnostics GmbH	CKIHA(cit)EIFDS(cit)GNPTVEC (cyclic)	1987 ACR criteria
Montes et al[35]	2011	Spain	Journal article	Case-control	ELISA	Plate: NR Antibody: NR	CKIHA(cit)EIFDS(cit)GNPTVEC (cyclic)	1987 ACR criteria
Lundberg et al[36]	2011	Sweden	Meeting abstract	Case-control	ELISA	Plate: NR Antibody: NR	NR	1987 ACR criteria
Snir et al[37]	2009	Sweden	Journal article	Case-control	ELISA	Plate: Nunc, Antibody: Jackson ImmunoResearch Laboratories	CKIHA(cit)EIFDS(cit)GNPTVEC (cyclic)	1987 ACR criteria
Lundberg[38]	2008	United Kingdom	Journal article	Case-control	ELISA	Plate: MaxiSorp Antibody: Hybridoma Reagent Laboratory	CKIHA(cit)EIFDS(cit)GNPTVEC (cyclic)	1987 ACR criteria

Abbreviations: CEP-1, citrullinated α-enolase peptide 1; cit, citrulline; NR, not reported.

and P values <.05 were considered to be indicative of the presence of a threshold effect. Meta-regression and subgroup analysis were performed to detect the potential source of heterogeneity, and P values <.05 were taken to be indicative of statistical significance. To detect publication bias, a Deek's funnel plot was employed, and P values <.10 were considered to be indicative of existing publication bias.

3 | RESULTS

3.1 | Search results

A total of 423 articles were searched in PubMed, Web of Science, Embase, Scopus, and Cochrane Library databases, of which 184 studies were duplicates and 145 were excluded after the title and abstract screening (Figure 1). Then, 94 studies underwent full-text review, of which 18 did not provide enough data to construct 2 × 2 contingency tables, 18 did not set up a control group, 12 reported that they included pre-RA patients who developed RA in the future, 8 did not detect anti-CEP 1 antibody in patients with RA, 6 were reported as meeting abstracts that had been officially published as papers, 3 reported that the patients with RA were not diagnosed according to the ACR or EULAR criteria, 3 were reviews, 1 reported that the number of included RA patients was <50, and 1 was an animal study. Therefore, 70 studies were excluded after full-text review. Finally, 24 articles met the criteria for inclusion in the meta-analysis.[15-38]

3.2 | Study characteristics and literature quality assessment

Tables 1 and 2 present the main characteristics of the 24 eligible studies. There were 17 380 RA patients, 1231 non-RA patients as the disease control group, and 6274 participants as the health control group. Among the 24 studies, 14 only used health donors as a control group,[15,18,20,21,23,26-28,30,33-37] 1 study only used non-RA patients as a control group,[24] and the other studies mixed health donors and non-RA patients as the control group.[16,17,19,22,25,29,31,32,38] Two studies reported as meeting abstracts,[26,36] and the other studies were reported as journal articles.[15-25,27-35,37,38] Most of the studies used ELISAs to detect the anti-CEP 1 antibody by commercial ELISA kits or a self-established ELISA method.[15-17,19,22,24,25,28,29,31-33,35-38] Two types of CEP-1 peptide sequences were used as coating antigens in the self-established ELISA methods: one was a CKIHA(cit)EIFDS(cit)GNPTVEC (cyclic) with C-terminal and N-terminal cysteines to facilitate cyclization in 6 included studies,[25,31,33,35,37,38] and another was a KIHA(cit)EIFDS(cit)GNPTVE without cysteine in 4 of the studies.[24,28,29,32] Detailed information about the self-established ELISA methods used in the included studies is presented in the supplementary files (see Table S1. [Appendix S2C]).

TABLE 2 The other characteristics of 24 eligible studies in this meta-analysis

Author	Published y	Age, y	RA number (F/M)	NRA (F/M)	Cut-off	TP	FP	TN	FN	SEN	SPE	RA duration	Anti-CEP+/anti-CCP−a/anti-CEP+/anti-CCP+b
Zhou et al[15]	2019	RA: 16-89 HC: 25-40	219/63	HC: 61/59	20 U/mL	184	16	104	98	65.2%	86.7%	NR	20.8% (10/48)/74.4% (174/234)
Ponikowska et al[16]	2019	RA: 49.0 ± 16.4 DC: 46.0 ± 19.3 HC: NR	35/16	DC:19/4 HC: 20	20 RU/mL	36	2	41	15	70.6%	95.3%	5.7 ± 3.5 mo	41.2% (7/17)/85.3% (29/34)
Liu et al[17]	2019	RA: 45-62 DC: 36-64 HC: 40-57	75/26	DC:27/19 HC: 51/28	20 RU/mL	62	11	114	39	61.4%	91.2%	MT:7 y (2, 16)	20.8% (5/24)/74.0% (57/77)
Rönnelid et al[18]	2018	RA: 18-70 DC: NR HC: NR	2825	HC:551	A specificity of 98.0% of healthy control	1332	11	540	1493	47.2%	98.0%	<1 y	NR
Meyer et al[19]	2018	RA: 48 DC: 43-45 HC: 46	61/14	DC: 46 HC: 19/10	46 RU/mL	54	18	57	21	72.0%	76.0%	MT:9 mo (12)	NR
Jonsson et al[20]	2018	RA: 51.5 ± 13.6 HC: 52.1 ± 9.2	131/86	HC: 57/37	A specificity of 98.0% of healthy control	140	1	93	77	64.5%	98.9%	<2 y	7.7% (3/39)/77.0% (137/178)
Alunno et al[21]	2018	RA: 62 ± 2 HC: NR	100	HC: 50	NR	45	1	49	55	45.0%	98.0%	12 ± 3 y	20.0% (7/35)/58.5% (38/65)
Alunno et al[22]	2018	RA: 61.7 ± 0.8 DC: NR HC: NR	196/56	DC: 97 HC: 50	20 U/mL	112	3	144	140	44.4%	98.0%	12.6 ± 0.6 y	17.6% (15/85)/58.1% (97/167)
Too et al (Malaysia)[23]	2017	RA: 18-70 HC: NR	1231	HC: 1625	A specificity of 98.0% of healthy control	283	33	1592	948	23.0%	98.0%	1.1 ± 1.8 y	NR
Too et al (Sweden)[23]	2017	RA:18-70 HC:NR	2858	HC: 578	A specificity of 98.0% of healthy control	1429	12	566	1429	50.0%	98.0%	<1 y	NR
Schwenzer et al[24]	2017	RA: NR DC: NR	287	DC: 330	A specificity of 98.0% of disease control	95	7	323	192	33.1%	97.9%	>10 y	NR
Cabrera-Villalba et al[25]	2017	RA: 54.7 ± 11.8 DC: 51.2 ± 11.3 HC: NR	34/20	DC: 34/20 HC: 64	A specificity of 98.0% of healthy control	22	18	100	32	40.7%	84.7%	3 years	NR
Brink et al[26]	2017	RA: 56.7 ± 14.0 HC: NR	692/330	HC: 477	A specificity of 98.0% of healthy control	549	10	467	473	53.7%	97.9%	<1 y	NR
Reed et al[27]	2016	RA:NR HC: NR	2836	HC: 373	A specificity of 98.0% of healthy control	1171	7	366	1665	41.3%	98.1%	NR	NR

(Continues)

TABLE 2 (Continued)

Author	Published y	Age, y	RA number (F/M)	NRA (F/M)	Cut-off	TP	FP	TN	FN	SEN	SPE	RA duration	Anti-CEP+/anti-CCP−[a]/anti-CEP+/anti-CCP+[b]
Choi et al[28]	2016	RA:58.2 ± 12.0 HC: 58.2 ± 11.6	231/33	HC: 77/11	A specificity of 95.0% of healthy control	46	4	84	218	17.4%	95.5%	13.8 ± 9.8 y	NR
Quirke et al[29]	2015	RA: NR DC: NR HC: NR	72/28	DC: 148/61 HC: 58/21	A specificity of 95.0% of healthy control	42	10	278	58	42.0%	96.5%	NR	NR
Kokkonen et al[30]	2015	RA: 56.5 HC: 50.3	199	HC: 574	A specificity of 97.0% of healthy control	134	35	539	65	67.3%	93.9%	MT:7.2 mo (4.7, 10.6)	NR
Umeda et al[31]	2013	RA: 16-84 DC: 15-84 HC: 18-55	158/50	DC:187/15 HC: 84/90	Mean + 3SD	92	4	372	116	44.2%	98.9%	NR	10.3% (3/29)/49.7% (89/179)
Goules et al[32]	2013	RA: NR DC: NR HC: NR	141	DC: 114 HC: 100	Mean + 2SD	53	9	205	88	37.6%	95.8%	NR	NR
Montes et al[33]	2012	RA: >55 HC: >55	404/117	HC: 173	A specificity of 98.0% of healthy control	117	3	170	404	22.5%	98.2%	MT: 18 y (10, 25)	NR
Hansson et al[34]	2012	RA: 18-70 HC: NR	927	HC: 461	A specificity of 98.0% of healthy control	365	10	451	562	39.4%	97.8%	<1 y	0% (0/526)/91.0% (365/401)
Montes et al[35]	2011	RA: ≥55 HC: ≥55	451	HC: 173	Mean + 3 SD	121	0	173	330	26.8%	100.0%	NR	12.5% (16/128)/32.6% (103/316)
Lundberg et al[36]	2011	RA: NR HC: NR	1985	HC: 150	A specificity of 98.0% of healthy control	714	3	147	1271	36.0%	98.0%	<1 y	NR
Snir et al[37]	2009	RA: 21-86 HC: 24-82	238/53	HC: 81/19	A specificity of 99.0% of healthy control	120	1	99	171	41.2%	99.0%	NR	1.2% (1/81)/56.7% (119/210)
Lundberg et al[38]	2008	RA: NR DC:NR HC:NR	102	DC: 110 HC: 92	OD values above 0.1	37	5	197	65	36.3%	97.5%	NR	23.3% (7/30)/41.7% (30/72)

Abbreviations: 2SD/3SD, the cut-off for a positive response as the mean plus 2/3 times the SD of the specificity anti-CEP 1 reactivity of the healthy control; Anti-CEP 1, anti-citrullinated α-enolase peptide 1; DC, disease control; F, female; FN, false negative; FP, false positive; HC, healthy control; M, male; MT, median time; NR, not reported; NRA, non-RA patients; OD, optical density; RA, rheumatoid arthritis; SEN, sensitivity; SPE, specificity; TN, true negative; TP, true positive.

[a]The prevalence of anti-CEP 1 antibody in anti-CCP antibody negative RA patients.

[b]The prevalence of anti-CEP 1 antibody in anti-CCP antibody-positive RA patients.

FIGURE 2 Literature quality assessment by using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool for eligible studies

The results of the included literature quality assessment are shown in Figure 2. We found a relatively high risk of bias or unclear risk of bias for patient selection because most of the articles did not specifically explain the sampling method of the included patients and it was difficult to achieve a consecutive or random sample of patients. A few studies indicated a high risk bias in flow and timing, because not all of the included patients in these studies underwent the anti-CEP 1 antibody test. However, regarding applicability, all studies had a low risk of bias.

3.3 | Diagnostic value of anti-CEP 1 antibody to RA

Among the 24 studies included in the meta-analysis, the sensitivity of the anti-CEP 1 antibody to RA ranged 17.4%-72.0%, and the specificity of the anti-CEP 1 antibody to RA ranged 76%-100%. Eleven studies out of 24 included studies showed the prevalence of the anti-CEP 1 antibody in the anti-CCP antibody negative RA patients or anti-CCP antibody-positive RA patients,[15-17,20-22,31,34,35,37,38] and the prevalences of the anti-CEP 1 antibody in the 2 groups of RA patients were 0%-41.2% and 32.6%-91.0%, respectively. The pooled sensitivity and specificity were 44% (95% CI: 38%-51%, $P < .0001$, $I^2 = 97.02\%$) and 97% (95% CI: 96%-98%, $P < .0001$, $I^2 = 92.77\%$), respectively (Figure 3A,B). Therefore, there was a significant heterogeneity among the studies. The pooled PLR was 14.81 (95% CI: 10.66-20.57, $P < .0001$, $I^2 = 81.29\%$), and the pooled NLR was 0.57 (95% CI: 0.52-0.64, $P < .0001$, $I^2 = 97.15\%$). The pooled PPV and NPV of the anti-CEP 1 antibody were 0.96 (95% CI: 0.95-0.97, $P < .0001$, $I^2 = 88.70\%$) and 0.53 (95% CI: 0.43-0.63, $P < .0001$, $I^2 = 99.60\%$), respectively. The pooled DOR was 25.83 (95% CI: 18.43-36.20), and the SROC showed that the AUC was 0.86 (95% CI: 0.82-0.88) (Figure 3C).

3.4 | Meta-regression and subgroup analysis

First, we evaluated the included studies to determine if there was an existing threshold effect. The Spearman's correlation coefficient for the anti-CEP 1 antibody was 0.342 ($P = .094$), indicating that there was no threshold effect.

However, there was substantial heterogeneity among the included studies, so we performed a meta-regression to explore the sources of heterogeneity. The following covariates were tested: the control group (healthy control and included disease control), diagnostic criteria for RA (the 1987 ACR criteria and 2010 ACR/EULAR criteria), and detection method for anti-CEP 1 antibody (commercial ELISA kit and non-commercial ELISA kit). Consequently, the control group and diagnostic criteria for RA were identified as significant contributors to the heterogeneity for specificity (both $P < .001$), whereas the detection method for RA contributed to the heterogeneity for sensitivity and specificity ($P < .05$ and $P < .001$, respectively) (Figure 4).

Furthermore, all covariates were subjected to subgroup analysis. In the controls subgroup, the group with healthy controls had

a sensitivity of 42% (95% CI: 34%-50%, $P < .0001$, $I^2 = 98.12\%$), a specificity of 98% (95% CI: 97%-98%, $P < .0001$, $I^2 = 88.57\%$), and an AUC of 0.92 (95% CI: 0.89-0.94); the group including the disease controls had a sensitivity of 48% (95% CI: 40%-56%, $P < .0001$, $I^2 = 87.15\%$), a specificity of 95% (95% CI: 91%-98%, $P < .0001$, $I^2 = 93.24\%$), and an AUC of 0.78 (95% CI: 0.74-0.82). In the diagnostic criteria for the RA subgroup, the group diagnosed with RA according to the 1987 ACR criteria had a sensitivity of 41% (95% CI: 35%-48%, $P < .0001$, $I^2 = 97.51\%$), a specificity of 97% (95% CI: 96%-98%, $P < .0001$, $I^2 = 92.80\%$), and an AUC of 0.84 (95% CI: 0.80-0.87); and the group diagnosed with RA according to the 2010 ACR/EULAR criteria had a sensitivity of 57% (95% CI: 47%-67%, $P < .0001$, $I^2 = 90.55\%$), a specificity of 96% (95% CI: 91%-98%, $P < .0001$, $I^2 = 87.35\%$), and an AUC of 0.86 (95% CI: 0.83-0.89). In the detection method for RA subgroup, the group that detected the anti-CEP 1 antibody by using a commercial ELISA kit had a sensitivity of 59% (95% CI: 50%-68%, $P < .0001$, $I^2 = 88.04\%$), a specificity of 93% (95% CI: 85%-97%, $P < .0001$, $I^2 = 86.42\%$), and an AUC of 0.79 (95% CI: 0.76-0.83); the group that detected the anti-CEP 1 antibody by using a home-made ELISA kit had a sensitivity of 33% (95% CI: 28%-39%, $P < .0001$, $I^2 = 90.35\%$), a specificity of 98% (95% CI: 96%-99%, $P < .0001$, $I^2 = 87.07\%$), and an AUC of 0.70 (95% CI: 0.66-0.74); and the group that detected the anti-CEP 1 antibody by using microarray had a sensitivity of 48% (95% CI: 38%-58%, $P < .0001$, $I^2 = 98.34\%$), a specificity was 97% (95% CI: 96%-98%, $P < .0001$, $I^2 = 89.46\%$), and an AUC of 0.95 (95% CI: 0.93-0.97). To explore the different antigen sources and to determine if they affected the diagnostic performance of the home-made ELISA kit, we performed an additional subgroup analysis. The group using a CEP-1 sequence with C-terminal and N-terminal cysteines had a sensitivity of 35% (95% CI: 28%-42%, $P < .0001$, $I^2 = 91.04\%$), a specificity of 98% (95% CI: 95%-100%, $P < .0001$, $I^2 = 93.73\%$), and an AUC of 0.62 (95% CI: 0.57-0.66). The group using a CEP-1 sequence without cysteines had a sensitivity of 31% (95% CI: 22%-42%, $P < .0001$, $I^2 = 90.57\%$), a specificity of 97% (95% CI: 95%-100%, $P = .48$, $I^2 = 0\%$), and an AUC of 0.96 (95% CI: 0.94-0.98). Other details of the summary diagnostic index are shown in the supplementary files (see Table S2. [Appendix S2D]).

3.5 | Publication bias and sensitivity analysis

The Deeks' funnel plot asymmetry test showed that no publication bias was observed ($P = .65$) (see Figure S1 [Appendix S2E]). The sensitivity analysis, excluding each article to perform the meta-analysis again, indicated that the meta-analysis results were stable (See Figure S2 [Appendix S2F]).

4 | DISCUSSION

Patients with RA who receive early successful treatment can achieve effective remission and possibly prevent onset of extra-articular
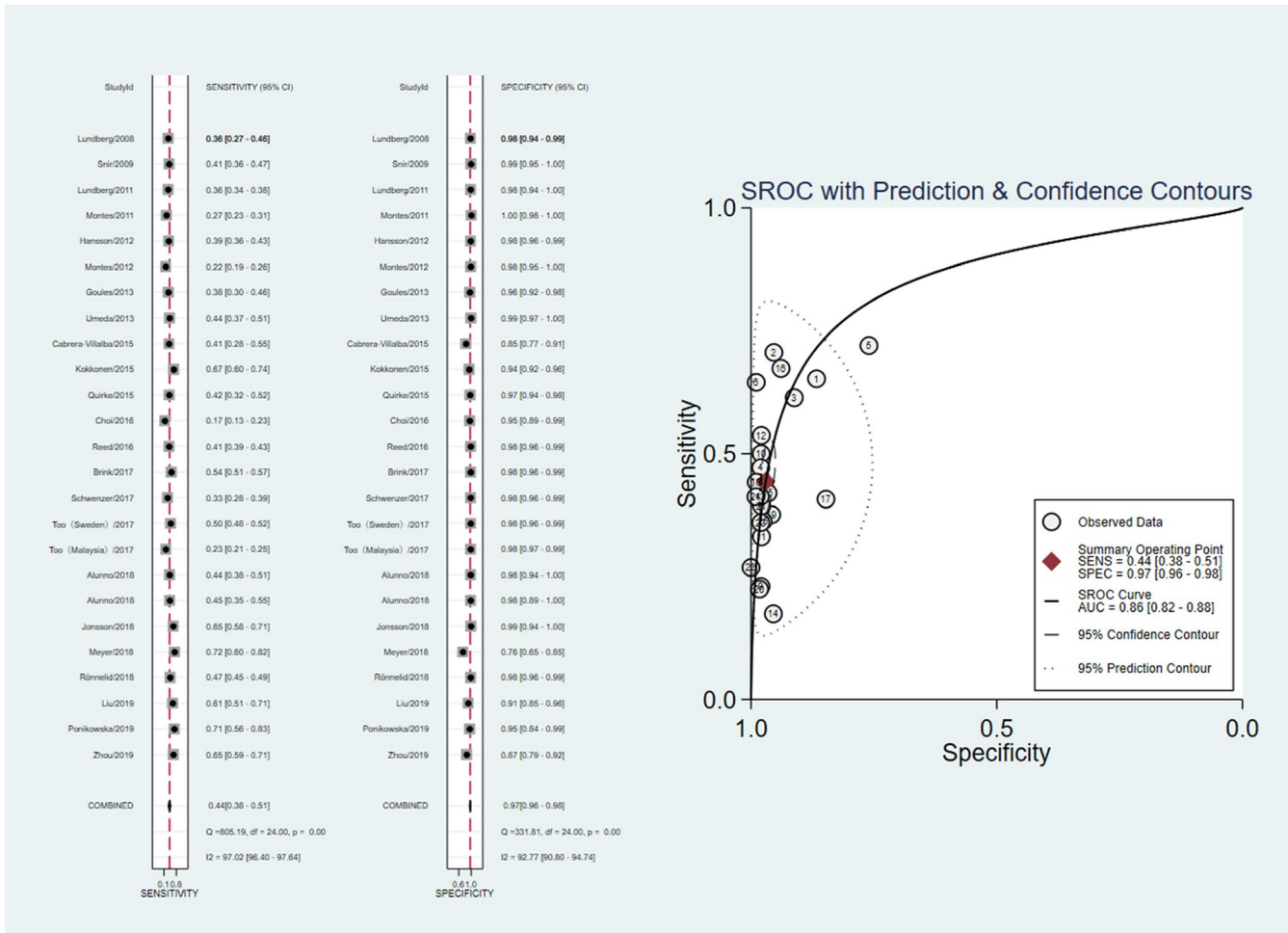
FIGURE 3 Forest plots and summary receiver operating characteristic curves of the anti-citrullinated α-enolase peptide 1 (anti-CEP 1) for rheumatoid arthritis (RA). (A) Sensitivity forest plot; (B) specificity forest plot; (C) summary receiver operating characteristic curve

manifestations. Therefore, it is necessary to identify RA as soon as possible. Many ACPAs have shown good performance for early diagnosis of RA.[39] The anti-CEP 1 antibody is one of the ACPAs that can be detected even before the onset of RA and may be involved at the beginning of RA.[40] Thus, detection of the anti-CEP 1 antibody may contribute to recognition of patients in early phase RA.

To our knowledge, this meta-analysis is the first to investigate the diagnostic value of anti-CEP 1 antibody for RA, with a total of 24 studies included. The potential diagnostic value of the anti-CEP 1 antibody for RA was mainly reflected by its predominant specificity (97%) and PLR (14.81), indicating that the subjects who tested positive for the anti-CEP 1 antibody had a 14.81-fold chance of developing RA relative to the chance for the subjects who tested negative. Therefore, the high PLR of the anti-CEP 1 antibody shows that a positive anti-CEP 1 antibody result has good accuracy for identifying subjects who have RA. However, the diagnostic value of the anti-CEP 1 antibody for RA was limited by its lower sensitivity (44%) and insufficiently low NLR (0.57), which suggests that the subjects who were suspected to have RA but tested negative for the anti-CEP 1 antibody could not be excluded from having RA. The higher pooled PPV (0.96) and lower pooled NPV (0.53) also indicated that

subjects with the anti-CEP 1 antibody had a higher probability of RA and those without the anti-CEP 1 antibody could not be ruled out as having RA and that the predicted values could be affected by disease prevalence.[41] Therefore, more studies are needed to confirm this result by adjusting for the prevalence of RA. The DOR (25.83) revealed that the anti-CEP 1 antibody was very helpful for diagnosing RA, and the AUC of 0.86 indicated moderate performance of the anti-CEP 1 antibody for diagnosing RA.

We observed high heterogeneity among the included studies. Therefore, we tested several factors that may influence heterogeneity by performing meta-regression and subgroup analysis. The meta-regression analysis indicated that the control group, diagnostic criteria for RA, and detection method for the anti-CEP 1 antibody were significant contributors to the heterogeneity for specificity, whereas the subgroup analysis suggested that the heterogeneity values for each of the specificity of 3 factors were not significantly different (range of I^2: 86.42%-93.24%) and the specificity was higher than 93%. Additionally, the subgroup analysis indicated that the group diagnosed with RA according to the 2010 ACR/EULAR criteria had a sensitivity (57%) that was about 16% higher than that of the group diagnosed with RA according to the

Univariable Meta-regression & Subgroup Analyses
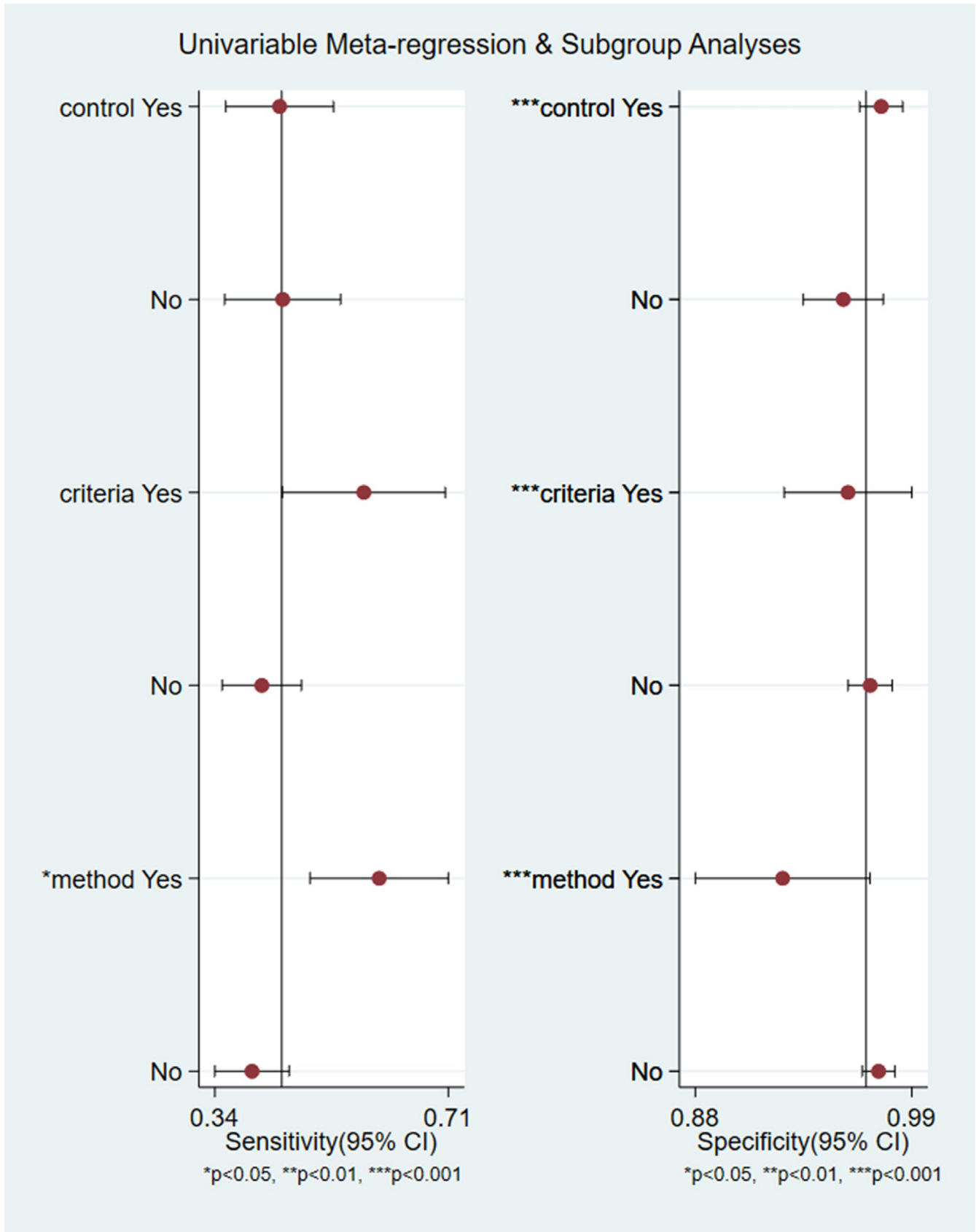
FIGURE 4 Univariable meta-regression and subgroup analysis of 3 covariates (control group, diagnostic criteria for rheumatoid arthritis (RA), method for detection of the anti-citrullinated α-enolase peptide 1 [anti-CEP 1] antibody)

1987 ACR criteria (41%). Clinically, there are 2 sets of criteria for diagnosing RA, and some studies have reported that the 2010 ACR/EULAR criteria have a higher sensitivity for identifying patients with RA but a lower specificity than that of the 1987 ACR criteria.[42-44] The results of the subgroup analysis were consistent with those of these published studies. The method for detecting the anti-CEP 1 antibody is a potential source of heterogeneity for sensitivity and specificity, which was tested by meta-regression and subgroup analysis. The group in which the anti-CEP 1 antibody was detected by using a commercial ELISA kit had the highest sensitivity (59%) of all subgroups, whereas the group in which the anti-CEP 1 antibody was detected by using a home-made ELISA kit had the lowest sensitivity (33%) among all subgroups, which indicated that the standardized commercial ELISA kit may improve the sensitivity for diagnosis of RA relative to that of the home-made ELISA kits that use a variety of materials and testing procedures. Additionally, the AUC of the commercial ELISA kit (0.79) was higher than that of the home-made ELISA kit (0.70), which indicated that detecting the anti-CEP 1 antibody by using the commercial ELISA kit had moderate diagnostic performance and was superior to that of the home-made ELISA kit. The group in which the anti-CEP 1 antibody was detected by using microarray had the highest AUC (0.95), which may be because only healthy donors were included in the control group. Therefore, the value of the microarray for detecting the anti-CEP 1 antibody should be investigated by additional studies that include a diseased-patient control. For the home-made ELISA kit, our data show that the sensitivity and specificity in the group using the CEP-1 peptide with C-terminal and N-terminal cysteines as coated antigens were slightly higher than the CEP-1 peptide without cysteines, but the AUC was lower. Therefore, an inconsistent antigen source may adversely affect the capability for detection of the anti-CEP 1 antibody and the diagnostic performance of home-made ELISA kits. Of note, although the meta-regression showed that 3 factors may contribute to the heterogeneity, the subgroup analysis did not explore the notable decrease in the I^2 value ($I^2 < 50\%$), which demonstrated that the combined effect of multiple factors may influence heterogeneity. However, some of our included studies had missing data, which prevented analysis of this issue.

To date, RA remains a clinical diagnosis and autoantibody tests only serve as an aid to clinical assessment because of the existence of patients with seronegative RA who are negative for both anti-CCP antibody and RF,[45] although anti-CCP antibody and RF are routinely detected as indicators for diagnosing RA. However, our meta-analysis indicated that due to the lower sensitivity of diagnosing RA and similar specificity relative to that of the anti-CCP antibody,[46] this demonstrated that anti-CEP 1 antibody detection is not superior to anti-CCP antibody detection for diagnosing RA. Nevertheless, some studies have indicated that detection of anti-CEP 1 antibody could identify patients with RA who had negative anti-CCP antibody tests. The available evidence shows that detection of the anti-CEP 1 antibody ranges 0%-41.2% in patients with RA who have negative anti-CCP antibody tests and from 32.6%-91.0% in patients with RA who have positive anti-CCP antibody

tests.[15-17,20-22,31,34,35,37,38] The results were significantly different due to variations in sample size, study design or patient ethnicity, so further study is needed to investigate the reasons for these variations. Additionally, the first presentation of the anti-CEP antibody can be earlier than the increase in the anti-CCP antibody.[47] Thus, the anti-CEP 1 antibody may have supplementary diagnostic value in patients with RA by combining analysis of the anti-CCP and anti-CEP 1 antibodies. Presumably, they may benefit from early and aggressive interventions. Some studies have indicated that patients with anti-CEP 1 antibody-positive RA are more likely to develop bone erosions or interstitial lung disease (ILD) than patients with anti-CCP antibody-positive RA,[17,22,35] although the anti-CCP antibody is also associated with an increased risk of developing bone erosion or ILD,[22] which suggests that the anti-CEP 1 antibody is a better ACPA than the anti-CCP antibody to predict the prognosis of RA. The anti-CEP 1 antibody, one of the ACPA that targets a true physiological protein, may participate in the pathogen of RA-associated clinical manifestations.[48] High levels of ACPAs are associated with the development of bone erosion in patients with RA.[7,49] ACPAs may contribute to activating osteoclasts through their Fc glycan interactions with Fc receptors on osteoclasts to promote osteoclast activation and subsequent development of bone erosion, which is dependent on antibody-mediated effect.[50] RA-associated ILD frequently appears in patients with positive ACPA and cigarette smoking, but the specific mechanism of the underlying association between lung injury and ACPA generation remains unclear.

There were several limitations in our meta-analysis. First, some published articles in other databases were not evaluated. Second, we used the QUADAS-2 tool to assess the quality of the included studies, and a high risk of bias and an unclear risk of bias for patient selection were observed because most of the included studies did not explain the sampling method used to select the included patients. Third, ACPAs are related to genetic factors and may be one of the sources of heterogeneity, but the included studies were missing a lot of data, so we did not consider this relationship when we performed the meta-regression and subgroup analysis. Fourth, the stage of RA in patients may affect the overall diagnostic value of the anti-CEP 1 antibody for RA, so more studies are needed to investigate the diagnostic value of the anti-CEP 1 antibody for RA in different disease stages. Fifth, some studies have detected the anti-CEP 1 antibody by performing in-house assays, which vary in performance because of varying factors, such as antigen source, plates, conditions of coating, and detection reagents, so the diagnostic value of the anti-CEP 1 antibody detected by in-house assays should be validated.

5 | CONCLUSIONS

In summary, this review shows that the anti-CEP 1 antibody has moderate RA diagnostic value with relatively low sensitivity and high specificity. Moreover, the use of commercial ELISA kits for detecting the anti-CEP 1 antibody increases the RA diagnostic sensitivity.

AUTHOR CONTRIBUTIONS

HL and YZ conceived and designed the study. HL and LB searched the literature, screened, and selected the eligible studies. HL and LB extracted data acquisition and conducted quality assessment. HL, CX, LL, SX, and HZ analyzed the data and made an interpretation. HL made a draft. All other authors reviewed it critically. All authors agree to be accountable for all aspects of the study in ensuring that questions related to the accuracy or integrity of any part of the study are appropriately investigated and resolved. All authors take full responsibility for the integrity of the study and approved the final manuscript as submitted.

DATA AVAILABILITY STATEMENT

All data relevant to the study are included in the article or uploaded as Supplementary Information.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.