Review

Diagnostic accuracy of anti-RA33 antibody for rheumatoid arthritis: systematic review and meta-analysis

X. Yang[1], M. Wang[1], X. Zhang[1], X. Li[1], G. Cai[1], Q. Xia[1], L. Wang[1], L. Xin[1], S. Xu[2], F. Pan[1]

[1]Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, Hefei, Anhui;
[2]Department of Rheumatism and Immunity, the First Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China.

Xiao Yang, MS
Mengmeng Wang, MS*
Xu Zhang, MS
Xiaona Li, MS*
Guoqi Cai, MS
Qing Xia, MS*
Li Wang, MS
Lihong Xin, MS
Shengqian Xu, MD
Faming Pan, PhD

*These authors contributed equally to this work and should be considered co-first authors.

Please address correspondence to:
Prof. Faming Pan,
Department of Epidemiology and Biostatistics,
School of Public Health,
Anhui Medical University,
81 Meishan Road,
Hefei, Anhui 230032, China.
E-mail: famingpan@ahmu.edu.cn

Received on September 29, 2015; accepted in revised form on January 7, 2016.

Clin Exp Rheumatol 2016; 34: 539-547.

Key word: rheumatoid arthritis, anti-RA33 antibody, biomarker, diagnostic value, meta-analysis

Funding: this study was supported by grants from the National Natural Science Foundation of China (30771849, 30972530, 81273169, 81573218, and 81571572).

Competing interests: none declared.

ABSTRACT

Objective. *The main purpose of this meta-analysis is to evaluate the diagnostic value of anti-RA33 antibody for rheumatoid arthritis.*

Methods. *In order to obtain eligible studies, a systematic literature search was performed on PubMed, Web of science, EBSCO, CNKI and CBM from January 2000 to September 2015. Quality Assessment of Diagnostic Accuracy Studies (QUADAS) was employed to assess the quality of the relevant studies. Meta-disc 1.4 and Stata 11.0 were adopted in this meta-analysis.*

Results. *After rigorous review, fifty studies were included in this study, which are all reliable to summarise the diagnostic value in this meta-analysis. The result of the analysis shows the pooled sensitivity is 0.33 (95% confidence interval (CI): 0.31–0.34) and the specificity is 0.90 (95% CI: 0.89–0.90), for the diagnosis of rheumatoid arthritis. Besides, the area under the summary ROC curve (AUC) is 0.6863.*

Conclusion. *The current evidence suggests that anti-RA33 antibody has high diagnostic specificity value for rheumatoid arthritis, which may be useful for the disease diagnostic application. To verify this conclusion, more prospective research on the diagnostic value of anti-RA33 antibody for rheumatoid arthritis are needed in the future.*

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease with unknown aetiology, characterised by inflammation and damage of joints affecting about 0.5% of the general population, which seriously influence the quality of life of people (1). The disease is usually progressive and systemic which causes damage of the synovial membranes of joints and eventually leads to bone and cartilage destruction. This destruction is generally irreversible along with persistent arthritis and gravis pain. In view of this, early diagnosis is fundamental, since early and aggressive interventions with effective biological treatments can alter the course of the disease (2).

Nowadays, tests for circulating auto antibodies are used throughout the world for the diagnosis of rheumatoid arthritis (RA) (3). The main auto antibodies markers for the diagnosis of RA include rheumatoid factors (RF), antikeratin antibodies (AKA), and antiperinuclear factor (APF) (4). Although, these serum biomarkers have diagnostic value for RA, they still have some deficiencies, *e.g.* the rheumatoid factor (RF) has been identified in other connective tissue diseases and in elderly individuals, which is lack of specificity (5). Therefore, some novel serological biomarkers are strongly needed to further improve the early diagnosis rate (6).

In 1989, an autoantibody directed to a protein with a molecular mass of approximately 33 KD, contained in nuclear extracts from HeLa cells, was detected in RA sera and named RA33 (7). Protein sequencing revealed that RA33 was identical to the heterogeneous nucleoriboprotein (hnRNP) A2 (8), which is found to have excessive expression in inflamed synovial tissues, but very low expression in normal joints, so that anti-RA33 antibody has been described as highly specific antinuclear antibody for RA (9). Previous studies reported that 29% of patients with early RA (<3 months disease duration) had been anti-RA33 positive at initial evaluation. Furthermore, none of the anti-RA33 positive patients having RF at initial diagnosis was noted (4, 10). This evidence indicates that anti-RA33 may be of significant value and relevance inearly diagnosis of RA. Therefore, we perform this meta-analysis to summarise

the published data on the sensitivity, specificity, likelihood ratios, and diagnostic odds ratio and to obtain further evidence to verify whether anti-RA33 antibody has diagnostic value for RA.

Materials and methods

Literature search strategy

The search term anti-RA33 antibody, anti-RA33 antibodies, autoantibody to heterogeneous nuclear ribonucleoprotein-A2, auto antibodies to heterogeneous nuclear ribonucleoprotein-A2 and rheumatoid arthritis were used to search for articles published in PubMed, Web of science, EBSCO, CNKI and CBM database, published during the period between January 2000 to September 2015. To obtain additional relevant articles, we scanned conference summaries and reference lists of articles identified in the initial search and even contacted authors to get additional information if necessary.

Inclusion and exclusion criteria

All enrolled studies met the following criteria:

1. Used the 1987 revised American College of Rheumatology (ACR) criteria as the reference diagnose standard for RA (11).
2. Studies that include anti-RA33 antibody and a diagnosis of RA
3. Study samples only retrieved on humans.
4. Provided enough data to allow calculation of sensitivity and specificity for the diagnosis of RA.
5. Only the most recent and large sample size study was included in the case of duplicated publications.

Date extraction

Relevant data were extracted by two reviewers (X. Yang and M. Wang) independently and disagreement was resolved by a third reviewer (L. Wang). The following data were collected from each study: first author, year of publication, publication language, the detection methods, numbers of cases and controls, control participants information, the true positives results (TP), false positives results (FP), false negatives results (FN), true negatives results (TN), sensitivity and specificity.

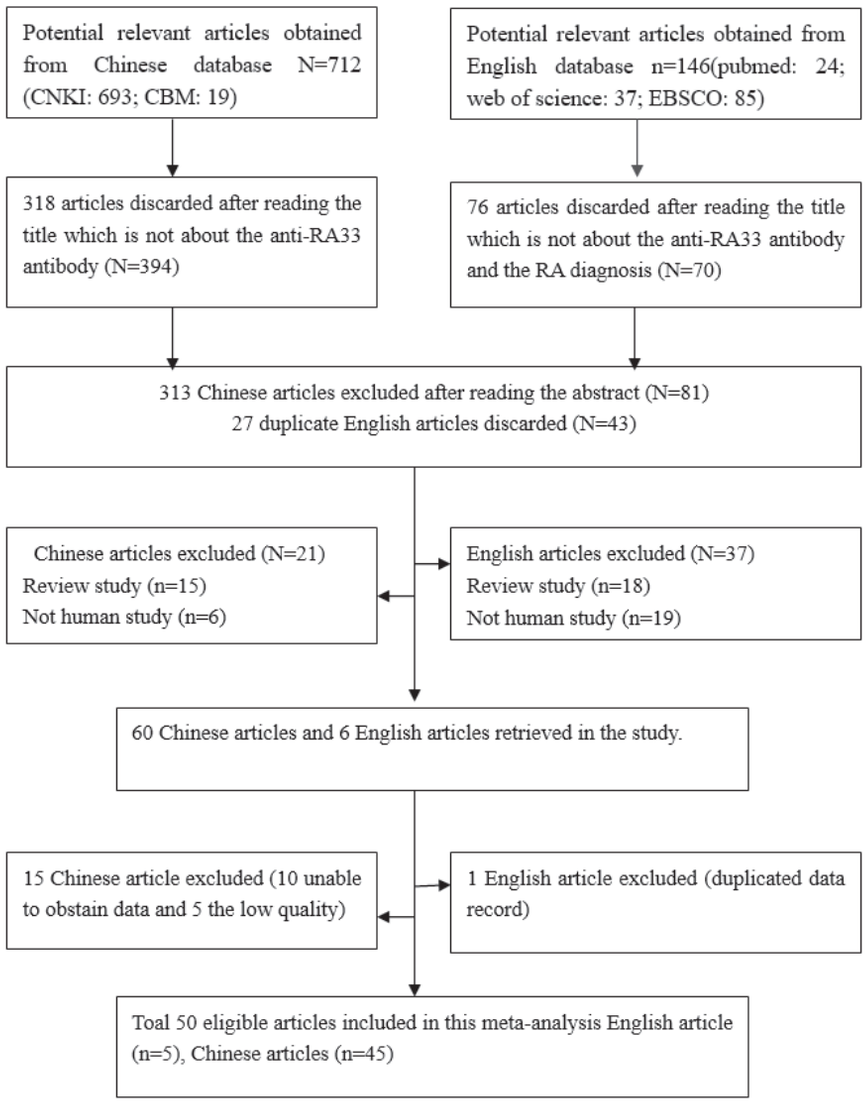

Fig. 1. Flow diagram for studies retrieved through the searching and selection processes.

Assessment of study quality

Two investigators independently assessed the methodological quality of each study by using 14 standard items from the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool (12), which is specifically developed for systematic reviews of diagnostic accuracy studies. Each question should answer with "yes" "no" or "unclear", of which "yes" obtains one score, "no" and "unclear" will obtain a score of zero. Any item discrepancies were resolved through discussion.

Statistical analysis

In this study, standard methods recommended for diagnostic accuracy meta-analysis were used (13). Basic data (TP, FP, FN,TN) collecting from each study was work up into one table, so that it's easy to gain sensitivity and specificity, and random effect model was adopted to combine sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-) and diagnostic odds ratio (DOR). In this study, 0.5 was added to each number for the value of zero occurring in the table, which was suggested by Cox (14). In addition, receiver operating characteristic (SROC) curve was constructed to manifest the summarised diagnostic rate (15). Each point of the curve represented one independent study. The area under the curve represented the overall diagnostic value, of which 1 indicates perfect discriminatory (16). Chi-square and I^2

Table I. Characteristics of studies about the anti-RA33 antibodies included in this meta analysis.

First author [study ref]	Time	Language	Detection method	Patients (control)	Women (%)	Mean or median age	Age range	Control participants
Li Hongbin (20)	2000	Chinese	western blot	128 (245)	82.8	41	19-75	Other rheumatic diseases (n=245)
Wang Yi (21)	2003	Chinese	ELISA	43 (68)	NA	NA	NA	Other rheumatic diseases (n=23), healthy persons (n=45)
Cui Tianpeng (22)	2003	Chinese	ELISA	46 (84)	89.1	35.1	NA	Other rheumatic diseases(n=54), healthy persons (n=30)
Yang Ling (23)	2003	Chinese	ELISA	179 (377)	NA	NA	NA	UIA (n=59), Other rheumatic diseases (n=278), healthy persons (n=40)
Zhong Liangyin (24)	2004	Chinese	ELISA	105 (111)	65.7	NA	27-67	Other rheumatic diseases (n=40), other non-rheumatic disease (n=35), healthy persons (n=36)
Zhang Hua (25)	2004	Chinese	ELISA	108 (185)	NA	NA	NA	Other rheumatic diseases (n=87), healthy persons (n=98)
Mei Xun (26)	2004	Chinese	ELISA	43 (88)	58.1	NA	23-72	Other rheumatic diseases (n=55), healthy persons (n=33)
Cheng Pengfei (27)	2005	Chinese	ELISA	68 (138)	76.5	42.3	NA	Other rheumatic diseases(n=98), healthy persons (n=40)
Liu Xueming (28)	2005	Chinese	western blot	100 (190)	NA	NA	NA	Other rheumatic diseases(n=166), healthy persons (n=24)
Chen Yanjie (29)	2005	Chinese	ELISA	31 (80)	63.3	41	28-81	UIA (n=30), Other rheumatic diseases (n=30), healthy persons (n=20)
Lei Xiaomei (30)	2005	Chinese	ELISA	104 (115)	83.7	54	41-78	Other rheumatic diseases (n=75), healthy persons (n=40)
Chen Linjie (31)	2005	Chinese	ELISA	124 (211)	75	NA	25-67	UIA (n=60), other rheumatic disease (n=151)
V PK Nell (32)	2005	English	western blot	102 (98)	NA	NA	NA	Other rheumatic diseases (n=98)
Li Shirong (33)	2006	Chinese	ELISA	42 (100)	73.8	36.3	16-71	UIA (n=21), Other rheumatic diseases (n=23), healthy persons (n=56)
Chen Minjing (34)	2006	Chinese	ELISA	250 (248)	66	48	19-72	Other rheumatic diseases (n=198), healthy persons (n=50)
Guo Yufan (35)	2006	Chinese	ELISA	94 (127)	72.3	NA	18-83	Other rheumatic diseases (n=97), healthy persons (n=30)
Gu Furong (36)	2007	Chinese	ELISA	118 (156)	71.2	38.5	20-74	Other rheumatic diseases (n=86), healthy persons (n=30)
Dai Liqun (37)	2007	Chinese	ELISA	47 (110)	76.6	36.3	16-61	UIA (n=25), Other rheumatic diseases (n=29), healthy persons (n=56)
Wang Li (38)	2007	Chinese	ELISA	254 (312)	77.2	41.4	25-75	Other rheumatic diseases (n=282), healthy persons (n=30)
Duan Falan (39)	2008	Chinese	ELISA	88 (100)	70.4	36.3	16-61	Other rheumatic diseases (n=50), healthy persons (n=50)
Wang Liping (40)	2008	Chinese	ELISA	47 (110)	76.6	36.3	16-61	UIA (n=25), Other rheumatic diseases (n=29), healthy persons (n=56)
Ji Chunmei (41)	2008	Chinese	ELISA	120 (155)	69.2	54	41-78	Other rheumatic diseases (n=115), healthy persons (n=40)
Wang Chunyan (42)	2008	Chinese	ELISA	75 (94)	72	39.9	17-82	Other rheumatic diseases (n=54),other non-rheumatic disease (n=40)
Zhong Guixiang (43)	2008	Chinese	ELISA	90 (120)	88.9	38	25-66	Other rheumatic diseases (n=80), healthy persons (n=40)
Zeng Huiqiong (44)	2008	Chinese	ELISA	78 (115)	93.6	37	22-61	Other rheumatic diseases (n=50), healthy persons (n=65)
Yu Yan (45)	2009	Chinese	ELISA	35 (30)	54.3	55.4	19-77	healthy persons (n=30)
Zhang Wei (46)	2009	Chinese	ELISA	60 (68)	60	47.9	23-71	Other rheumatic diseases (n=38), healthy persons (n=30)
Guo Xinghua (47)	2009	Chinese	ELISA	241 (617)	NA	NA	NA	Other rheumatic diseases (n=377), healthy persons (n=240)
He Zhixiang (48)	2010	Chinese	ELISA	63 (97)	71.4	57	14-86	Other rheumatic diseases (n=67), healthy persons (n=30)
Ou Yangyi (49)	2010	Chinese	ELISA	235 (50)	66.8	43	11-79	healthy persons (n=50)
Zhang Guoqing (50)	2010	Chinese	ELISA	60	63.3	46.7	21-70	Other rheumatic diseases (n=38), healthy persons (n=30)
Wang Yuhui (51)	2010	Chinese	ELISA	80 (92)	58.8	NA	19-72	Other rheumatic diseases (n=52), healthy persons (n=40)
Le Huabang (52)	2011	Chinese	ELISA	45 (90)	71.1	36.8	17-60	Other rheumatic diseases (n=45), healthy persons (n=45)
Zhu Hongxue (53)	2011	Chinese	ELISA	65 (100)	60	38.5	21-74	Other rheumatic diseases (n=50), healthy persons (n=50)
Qin Wangsen (54)	2011	Chinese	ELISA	82 (100)	82.9	NA	22-71	Other rheumatic diseases (n=50), healthy persons (n=50)
Zhang Ying (55)	2011	Chinese	ELISA	98 (65)	60.2	NA	18-75	healthy persons (n=65)
Zhang Wenlan (56)	2011	Chinese	ELISA	179 (216)	70.4	37	22-72	Other rheumatic diseases (n=156), healthy persons (n=60)
Yao Yanhong (57)	2012	Chinese	ELISA	78 (122)	66.7	46	20-65	Other rheumatic diseases (n=42), healthy persons (n=80)
Chen Haoquan (58)	2013	Chinese	ELISA	95 (100)	63.2	NA	23-72	Other rheumatic diseases (n=50), healthy persons (n=50)
Chen Chao (59)	2013	Chinese	ELISA	80 (130)	0.775	NA	36-83	Other rheumatic diseases (n=50), healthy persons (n=80)
Niu Ruibing (60)	2013	Chinese	ELISA	78 (102)	NA	NA	NA	Other rheumatic diseases (n=72), healthy persons (n=30)
Mohammed Marrof Al-Ani(61)	2013	English	ELISA	50 (40)	84	NA	18-67	Other rheumatic diseases (n=40)
Yongmei Zhou (62)	2013	English	ELISA	305 (50)	74.4	41	9-79	Healthy persons (n=50)
Zhong Ruifen (63)	2014	Chinese	ELISA	130 (120)	33.8	53.1	37-68	Healthy persons (n=120)
Chen Shuang (64)	2014	Chinese	ELISA	103 (145)	72.8	NA	40-76	Other rheumatic diseases (n=41), healthy persons (n=104)
Zheng Hongxia (65)	2014	Chinese	ELISA	93 (120)	75.3	NA	25-70	Other rheumatic diseases (n=60), healthy persons (n=60)
Gao Lixia (66)	2014	Chinese	ELISA	90 (105)	58.9	30.1	17-70	Other rheumatic diseases (n=105)
Mahin Lashkari (67)	2014	English	ELISA	43 (55)	NA	NA	30-49	Healthy persons (n=55)
Jamil A. Al-Mughales (68)	2015	English	ELISA	41 (60)	NA	NA	NA	Other rheumatic diseases (n=31), healthy persons (n =29)
Wang Ting (69)	2015	Chinese	ELISA	90 (147)	55.6	31	18-69	Other rheumatic diseases (n=107), healthy persons (n=40)

test were used to assess the heterogeneity in studies. A *p*-value less than 0.05 and I^2 more than 50% indicated the existence of significant heterogeneity (17, 18). Exploring the possible reasons of heterogeneity among the studies is an important part of meta-analysis (19), which consists of threshold and non-threshold heterogeneity. All statistical analyses were performed using STATA 11.0 (Stata-Corp, College Station, TX, USA) and MetaDiSc (v. 1.4) software.

Results

Search results and characteristics of the studies

We identified 858 articles at the first literature search, of which 50 eligible articles (20-69) met the inclusion criterion and were included in this meta-analysis, finally. Among them, forty-five articles were in Chinese and five were in English. The detailed inclusion procedure is given in Fig. 1 and Table I summarises the characteristics of the included studies. There were about 5003 RA patients enrolled in this Meta analysis. The median number of RA patients in this meta-analysis was 89, of which their median age was 41 years old and the median proportion of women was 71.1%. Most of the studies included in the meta-analysis adopted the enzyme linked immunosorbent assay (ELISA) method to detect the anti-RA33 antibodies in the serum, of which only three articles used the Western blotting method. Besides, the participants of the control group were varied. Among these articles, six studies only used healthy individuals and four studies simply used the patients with other non-RA rheumatic disease. In the remaining studies, both healthy individuals and other rheumatic disease patients comprised the control group.

Study quality

The included studies satisfied at least 11 items of the 14 items using the QUADAS tool. Although none of the study met all the 14 standard items, most of studies achieved a score of 12, which could be reliable enough to summarise the result. The detailed quality information of each study is in the QUADAS scores in Table I.

Table I. *Continued*.

Study ref	QUADAS Scores	TP	FP	FN	TN	Sensitivity (%)	Specificity (%)
20	13	37	25	91	220	28.9	89.8
21	12	9	8	34	60	20.9	88.2
22	12	8	2	66	82	17.4	97.6
23	13	66	69	113	308	36.9	87.1
24	12	30	9	75	102	28.6	92
25	13	43	19	65	166	39.8	89.7
26	13	10	2	33	86	23.3	97.7
27	13	23	10	45	128	33.82	92.86
28	12	38	35	62	155	42.2	81.6
29	12	11	3	20	77	35.5	96
30	12	30	11	74	104	28.8	90.7
31	12	46	9	78	192	37.1	90.99
32	12	29	10	73	88	28.00	90.00
33	11	18	9	24	91	42.9	91.3
34	12	95	7	212	238	38	96
35	13	30	31	64	96	31.9	75.3
36	12	40	14	78	142	33.9	91
37	12	21	11	26	99	44.7	89.6
38	13	92	34	162	162	36.2	82.4
39	11	35	6	53	94	39.8	94
40	12	21	11	26	99	44.7	89.6
41	12	40	8	80	147	33.3	94.8
42	12	23	5	52	89	30.7	94.7
43	11	33	11	57	109	36.7	90.9
44	13	34	5	44	110	43.6	95.7
45	13	12	2	23	28	34.29	93.33
46	12	15	2	45	66	25	97.4
47	12	45	69	196	548	18.7	88.8
48	13	17	9	46	88	27	91
49	11	88	2	147	48	37.45	96
50	11	15	1	45	67	25	98.5
51	12	25	4	55	88	31.3	95.6
52	12	15	6	30	84	33.3	93.3
53	13	22	3	43	97	33.9	97
54	13	9	1	73	99	11	99
55	12	39	7	59	58	40	89.2
56	13	70	18	109	198	39.1	91.7
57	12	34	3	44	119	43.6	97.5
58	12	13	2	82	98	13.21	98
59	12	23	9	57	121	28.8	93
60	12	20	9	58	93	25.6	91.7
61	12	29	3	21	37	58	92.5
62	11	111	0	194	50	36.39	100
63	11	15	50	115	70	11.50	58.10
64	13	32	6	71	139	31.1	95.9
65	11	19	4	74	116	20.4	96.7
66	12	40	15	50	90	44.5	85.3
67	11	42	44	1	11	98.00	20.00
68	13	3	2	38	58	7.3	96.5
69	11	40	12	50	135	44.44	91.59

FN: false positive; FP: false positive; TN: true negative; TP: true positive; NA: not available.

Results of the meta-analysis

Sensitivity, specificity, positive likelihood ratio (PLR) and negative likelihood ratio (NLR) were adopted to evaluate the diagnostic accuracy of anti-RA33. Considering significant heterogeneity among the include studies, (sensitivity, I^2=83.1%; specificityI^2=88.9%, see Supplementary Fig. 1-2), random effect model was used to summarise the effect size.

The pooled diagnostic accuracy of anti-RA33.

We first analyse the pooled diagnostic accuracy of anti-RA33 antibody, which contained all the 50 articles. As Table II showed that the pooled sensitivity and

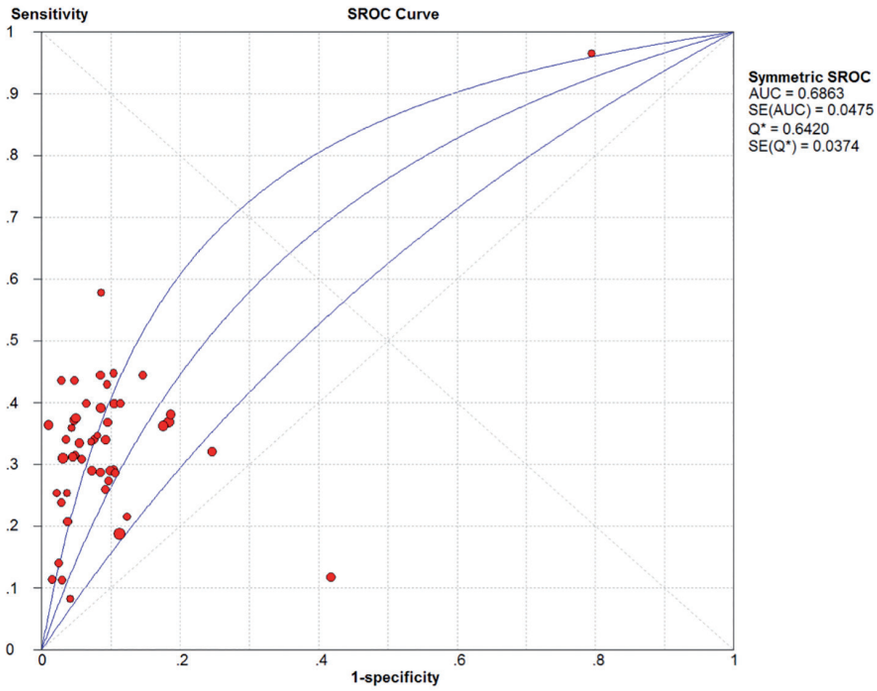

Fig. 2. Summary receiver operating characteristics (SROC) curve of all the studies for the diagnosis of RA through anti-RA33.

Table II. Pooled diagnostic performance.

Parameter	Result	95% CI	Heterogeneity chi-squared	*p*-value
pooled sensitivity	0.33	0.31-0.34	290.72	<0.001
pooled specificity	0.90	0.89-0.90	442.36	<0.001
pooled LR+	3.92	3.08-5.00	392.11	<0.001
pooled LR-	0.75	0.71-0.78	263.13	<0.001
DOR	5.49	4.27-7.06	222.31	<0.001
Spearman correlation coefficient	0.304			0.032
SROC				
AUC	0.6863			
Q*	0.6420			

LR+/–: positive/negative likelihood ratio, respectively; CI: confidence interval; DOR: diagnostic odds ratio; SROC: summary receiver operative curves; AUC: area under the SROC curve ; Q*: Q index.

specificity were 0.33 (95%CI: 0.31–0.34) and 0.90 (95%CI: 0.89–0.90), respectively, and the pooled PLR and NLR were 3.92 (95%CI: 3.08–5.00) and 0.75 (95%CI: 0.71–0.78). In this study, the diagnostic OR (DOR) was 5.49 (95%CI: 4.27–7.06), and the area under the SROC curve was 0.6863, with a Q value of 0.6420 (Fig. 2). The preliminary results are seen in Table II.

Heterogeneity test and exploration

As all the summary measures were significantly heterogeneous, we, thus, probed reasons for heterogeneity through subgroup analysis, which is also the important part job for the meta-analysis. The heterogeneity in this meta-analysis may result from two aspects, that is, the threshold effect and non-threshold effect. The threshold effect is due to different thresholds to define positive and negative test results, plotting the ROC plane of the sensitivity and specificity would be useful to identify the threshold effect, a curvilinear pattern indicates existing threshold effect exists. Besides, calculating the Spearman correlation coefficient between sensitivity and 1-specificity would also be helpful to test this threshold effect (70). In this study, the Spearman correlation coefficient of all the articles was 0.304, while *p*-value was 0.032, less than 0.05, which indicated the threshold effect heterogeneity (see Table II).

In view of the fact that the threshold effect heterogeneity existed in all the articles, we defined three subgroups on the basis of the publication language, detection method and the control participants. We made a separate subgroup meta-analysis three times according to the group situation. As shown in Table II, the *p*-value of the Spearman correlation coefficient indicated that the threshold effect heterogeneity was >0.05 with the development of the subgroup analysis, except for the ELISA detection method and the mixed participants control subgroup. In addition, in the Western blot and other rheumatic disease participants control subgroup, the Q-test of DOR showed that the *p*-value was 0.7601 and 0.1883, respectively (>0.05), which indicated the absence of non-threshold effect. It is shown in Fig. 3 that the sensitivity and specificity of the Western blot subgroup were 0.32 (95%CI: 0.27–0.37) and 0.87 (95%CI: 0.84–0.90), respectively. The area under the SROC curve was 0.5857, with a Q-value of 0.5645. Besides, the sensitivity and specificity of the other rheumatic disease participants control subgroup were 0.41 (95%CI: 0.35–0.46) and 0.93 (95%CI: 0.90–0.95), respectively. The area under the SROC curve was 0.7014, with a Q-value of 0.6540. (Fig. 4)

Publication bias assessment

The Deeks' funnel plots of the 50 included articles for detecting publication bias showed some asymmetry, indicating a potential publication bias (Supplementary Fig. 4).

Discussion

Rheumatoid arthritis (RA) is a chronic systematic autoimmune disease, which could cause severe arthropathy and joint destruction, and the disease being poorly controlled. As is known to us, the disease is a long-term inflammatory disorder and is not curable by drugs. Seeking more sensitive and specific early diagnosis tools will facilitate an earlier, aggressive treatment, which still remains challenging. At present, the diagnosis of RA is mainly based on

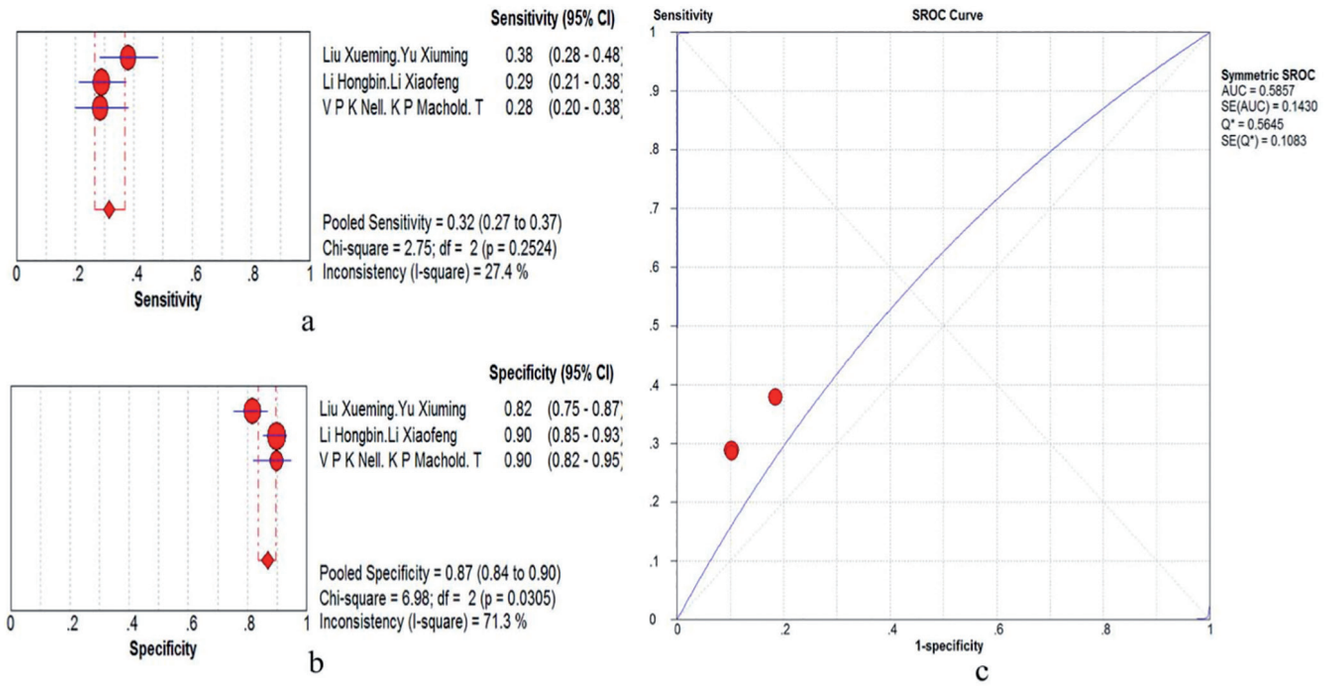

Fig. 3. The diagnostic indices for the diagnosis of RA using anti-RA33 antibody in the Western blot subgroup.
a: the sensitivity for the diagnosis of RA using anti-RA33; **b**: the specificity for the diagnosis of RA using anti-RA33; **c**: summary receiver operating characteristics (SROC) curve for the diagnosis of RA through anti-RA33.

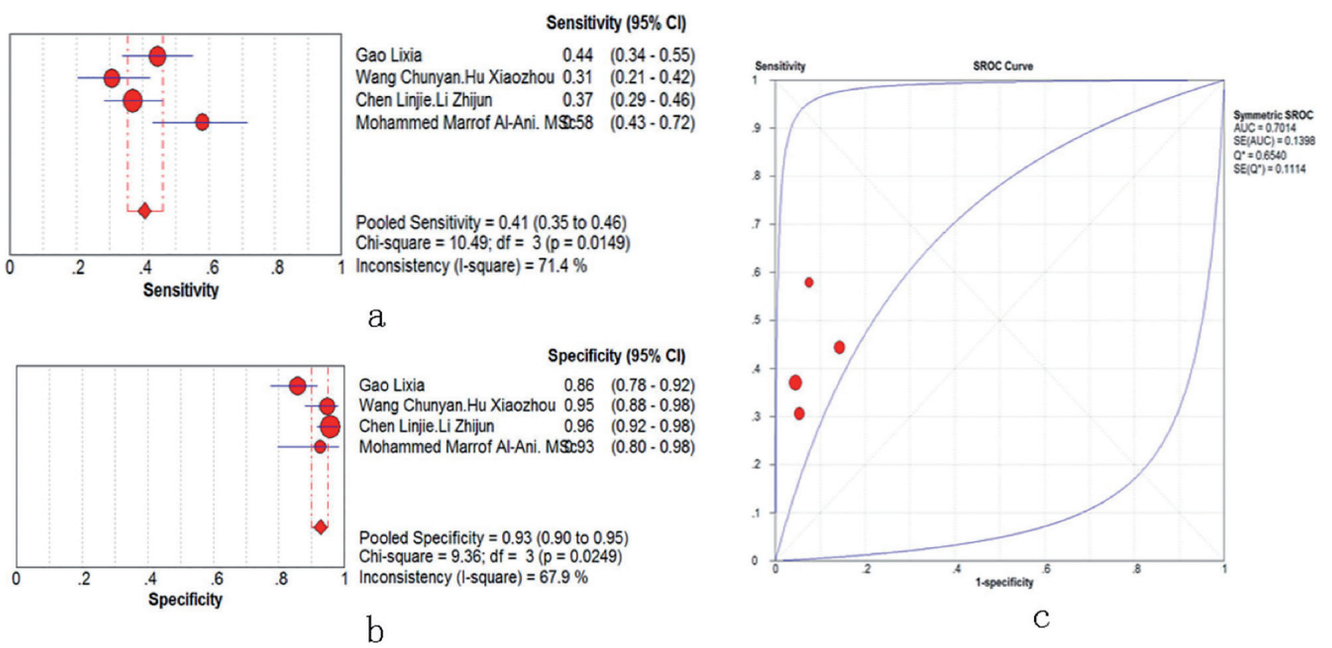

Fig. 4. The diagnostic indices for the diagnosis of RA using anti-RA33 antibody in the other rheumatic disease participants control subgroup.
a: the sensitivity for the diagnosis of RA using anti-RA33; **b**: the specificity for the diagnosis of RA using anti-RA33; **c**: summary receiver operating characteristics (SROC) curve for the diagnosis of RA through anti-RA33.

the anti-CCP antibody and RF, which has gained widely recognition by most rheumatologists (71, 72). However, the shortcomings are also known to us all, of which RF has low specificity, being identified in other connective tissue disease and healthy elderly individuals (5), the anti-CCP antibody positive is usually associated with severe disease like bone erosion (73, 74). Considering these points, it's urgent for us to explore novel serum biomarker for the early and accuracy diagnosis of RA. In recent years, research on anti-RA33 antibody for RA diagnosis has been frequently reported. Anti-RA33 antibody is directed to the heterogeneous nuclear ribonucleoprotein A2 (hnRNP-A2), a nuclear protein that is involved in mRNA splicing and transport (7, 8), which could be detected by ELISA and

Table III. Subgroup analysis of diagnostic accuracy variables.

Parameter	sens	spec	LR+	LR-	DOR	AUC	Q*	Spearman correlation coefficient
Publication language								
Chinese (n=45)								
Summary accuracy	0.32	0.91	4.14	0.75	5.73	0.5396	0.5297	0.294
95%CI	0.30-0.33	0.90-0.91	3.29-5.21	0.71-0.79	4.36-7.54			
Heterogeneity (*p*-value)	<0.001	<0.001	<0.001	<0.001	<0.001			0.050
English (n=5)								
Summary accuracy	0.40	0.80	3.78	0.69	6.62	0.7908	0.7279	0.500
95%CI	0.36-0.44	0.75-0.84	0.68-21.14	0.53-0.89	2.45-17.84			
Heterogeneity (*p*-value)	<0.001	<0.001	<0.001	<0.001	0.0542			0.391
Detection method								
ELISA (n=47)								
Summary accuracy	0.33	0.90	4.09	0.74	5.76	0.6989	0.6520	0.340
95%CI	0.31-0.34	0.89-0.91	3.14-5.33	0.71-0.78	4.39-7.56			
Heterogeneity (*p*-value)	<0.001	<0.001	<0.001	<0.001	<0.001			0.019
Western blotting(n=3)								
Summary accuracy	0.32	0.87	2.42	0.79	3.18	0.5857	0.5645	0.866
95%CI	0.27-0.37	0.84-0.90	1.85-3.18	0.73-0.85	2.24-4.51			
Heterogeneity (*p*-value)	0.2524	0.0305	0.5271	0.8979	0.7601			0.333
Healthy persons (n=6)								
Summary accuracy	0.36	0.71	2.45	0.75	4.84	0.7218	0.6704	0.257
95%CI	0.33-0.40	0.66-0.76	0.86-6.92	0.54-1.04	0.74-31.58			
Heterogeneity (*p*-value)	<0.001	<0.001	<0.001	<0.001	<0.001			0.623
Other rheumatic diseases (n=4)								
Summary accuracy	0.41	0.93	5.42	0.65	8.60	0.7014	0.654	0.600
95%CI	0.35-0.46	0.90-0.95	3.13-9.38	0.56-0.74	4.88-15.15			
Heterogeneity (*p*-value)	0.0149	0.0249	0.1047	0.0858	0.1883			0.400
The mixed group (n=37)								
Summary accuracy	0.31	0.91	4.38	0.75	6.07	0.6225	0.5925	0.407
95%CI	0.29-0.32	0.91-0.92	3.52-5.44	0.72-0.79	4.73-7.78			
Heterogeneity (*p*-value)	<0.001	<0.001	<0.001	<0.001	<0.001			0.012

Sens: sensitivity; spec: specificity; LR+/–: positive/negative likelihood ratio, respectively; CI: confidence interval; DOR: diagnostic odds ratio; SROC: summary receiver operative curves; AUC: area under the SROC curve; Q*: Q index.

immunoblotting (62). It has been reported that the antibody found by Hassfeld *et al*. (10), has been identified in 35% anticitrulline-negative RA, which is linked to the mild disease course of RA (75). Besides, the antibody might occur in the early stage of RA, and it has no association with RF, which could compensate for the deficiency of RF, especially when RF is negative (62). This meta-analysis aims to summarise the previous studies, then to provide up-to-date and comprehensive information to evaluate the overall diagnostic accuracy value of anti-RA33 antibody for the diagnosis of RA.

In the present study, the pooled sensitivity is 0.33 (95%CI: 0.31–0.34), which indicates the antibody has a moderate sensitivity, being consistent with the report from Ronnelid *et al*. (76), who showed its sensitivity ranges between 30%–50%. It is reported that anti-RA33 antibody has a high specificity, which is helpful in the diagnosis of RA patients who are anti-CCP and RF negative (61). The current study showed that the summarised estimate of specificity was 0.90 (95%CI: 0.89–0.90), which was not as high as the expected. A positive likelihood ratio of 3.92 suggested that patients with RA had a 3.92-fold higher chance of being anti-RA33 antibody tests positive compared with patients without RA, which manifested a potential role for anti-RA33 confirming RA. The diagnostic odds ratio (DOR) defined as the ratio of the odds of a true-positive to the odds of a false-positive, is another single indicator reflecting the test's performance accuracy that combines the sensitivity and specificity. The value of DOR ranges from 0 to infinity with higher values indicating better discriminatory test performance (77). SROC is usually used to summarise overall test performance and the area under the SROC curve (AUC) is calculated to evaluate accuracy of the selected indicator. To demonstrate excellent accuracy, the valve of AUC should be more than 0.97, an AUC of 0.75 to 0.92 is considered to be good (78, 79). In this meta-analysis, the DOR of the overall studies is 5.49 and the area under the SROC curve is 0.6863. Since the value is not high enough, it has only shown that the antibody could be a potential useful indicator, but further research is needed to verify it.

One main job of the meta-analysis was to explore the reasons for heterogeneity rather than simply summarise the estimate of effect size (19). The heterogeneity of the diagnostic study comprises threshold effect and non-threshold

effect. Threshold effect refers to the differences in sensitivities and specificities of the included studies accused by different cut-offs. At the preliminary meta-analysis, there actually exists the threshold effect heterogeneity in all the 50 included articles. In order to explore the heterogeneity and guarantee the reliability and stability of the results, the present studies carry out the subgroup analysis according to the publication language, detection method and the control participants, step by step. At the last analysis, even if both the threshold effect and non-threshold effect heterogeneity were eliminated, the value of all the diagnostic indexes fluctuated little. Thus, it was reliable to adopt the pooled results discussed above.

We established full-scale search strategy to carry out this meta-analysis, which is crucial for the quality of the results. Besides, the quality of most of the included articles is of a high level in term of QUADAS, that all the scores are higher than eleven. Although we tried to avoid the biases in the meta-analysis process, there were still several limitations in our study. Firstly, although we tried to scan all the studies on the diagnostic value of anti-RA33 antibody, a small amount of relevant literature may have been omitted. Secondly, most of the included articles were published in Chinese and the number of English articles was small, with just five studies, which mean that the results may not be applicable to the other populations, considering the possible race difference. Thirdly, because anti-RA33 antibody could be detected in several other connective tissue disorders, the control group of some included articles in this meta analysis was not only of normal controls, but also disease controls, which may make the result unreliable. However, in the present study, the result of subgroup analysis showed that the specificity of the antibody changed only a little, increasing from 0.90 to 0.94. Finally, due to the number limitation of the last subgroup, we were unable to detect whether the publication bias existed in the subgroup studies.

In conclusion, this meta-analysis was the first meta-analysis on anti-RA33 antibody for the diagnosis of RA, and it was shown that anti-RA33 antibody had considerable high specificity, which may be a potential useful marker for the early diagnosis of RA. In view of the deficiency of the meta-analysis, further study on this antibody is needed.

References

1. SCOTT DL, WOLFE F, HUIZINGA TWJ: Rheumatoid arthritis. *Lancet* 2010; 376: 1094-108.
2. DE VRIES-BOUWSTRA JK, DIJKMANS BA, BREEDVELD FC: Biologics in early rheumatoid arthritis. *Rheum Dis Clin North Am* 2005; 31: 745-62.
3. AHO K, PALUSUO T, KURKI P: Marker antibodies of rheumatoid arthritis: diagnostic and pathogenetic implications. *Semin Arthritis Rheum* 1994; 23: 379-87.
4. CORDONNIER C, MEYER O, PALAZZO E, DE BANDT M, ELIAS A, NICAISE P: Diagnostic value of anti-RA 33 antibody, antikeratin antibody, antiperinuclear factor and antinuclear antibody in early rheumatoid arthritis: Comparison with rheumatoid factor. *Br J Rheumatol* 1996; 35: 620-4.
5. VAN BOEKEL MA, VOSSENAAR ER, VAN DEN HOOGEN FH, VAN VENROOIJ WJ: Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value. *Arthritis Res* 2002; 4: 87-93.
6. TROUW LA, MAHLER M: Closing the serological gap: promising novel biomarkers for the early diagnosis of rheumatoid arthritis. *Autoimmun Rev* 2012; 12: 318-22.
7. HASSFELD WS, HARTMUTH GK *et al*.: Demonstration of a new antinuclear antibody (anti-RA33) that is highly specific for rheumatoid arthritis. *Arthritis Rheum* 1989; 32: 1515-20.
8. STEINER GH, SKRINER K *et al*.: Purification and partial sequencing of the nuclear autoantigen RA33 shows that it is indistinguishable from the A2 protein of the heterogeneous nuclear ribonucleoprotein complex. *J Clin-Invest* 1992; 90: 1061-6.
9. DUMORTIER H, MONNEAUX F, JAHN-SCHMID B *et al*.: B and T cell responses to the spliceosomal heterogeneous nuclear ribonucleoproteins A2 and B1 in normal and lupus mice. *J Immunol* 2000; 165: 2297-305.
10. HASSFELD WS, GRANINGER G, WITZMANN WG, SCHWEITZER H, SMOLEN JS: Autoantibody to the nuclear antigen RA33: a marker for early rheumatoid arthritis. *Br J Rheumatol* 1993; 32: 199-203.
11. ARNETT FC, EDWORTHY SM, BLOCH DA *et al*.: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.
12. WHITING P, RUTJES AWS, REITSMA JB, BOSSUYT PMM, KLEIJNEN J: The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Med Res Methodol* 2003; 3: 25.
13. DEVILLE WL, BUNTINX F, BOUTER LM *et al*.: Conducting systematic reviews of diagnostic studies: didactic guidelines. *BMC Med Res Methodol* 2002; 2: 9.
14. DR C: The analysis of binary data. London: Methuen, 1970.
15. GATSONIS C.A.PP: Meta-analysis of diagnostic and screening test accuracy evaluation: methodologic primer. *AJR Am J Roentgenol* 2006; 187: 271-81.
16. PAI MF, PAI LL, HUBBARD N, RILEY ALW, COLFORD JM, JR.: Diagnostic accuracy of nucleic acid amplification tests for tuberculous meningitis: a systematic review and meta-analysis. *Lancet Infect Dis* 2003; 3: 633-43.
17. DINNES JD, KIRBY J, RODERICK JP: A methodological review of how heterogeneity has been examined in systematic reviews of diagnostic test accuracy. *Health Technology Assessment* 2005; 9: 1-113, iii.
18. HIGGINS JPT, DEEKS SG, ALTMAN JJ, DG: Measuring inconsistency in meta-analyses. *BMJ* 2003; 327: 557-60.
19. PETITTI DB: Approaches to heterogeneity in meta-analysis. *Stat Med* 2001; 20: 3625-33.
20. LI H, LI X, GAN X: Special antibodies for the early diagnosis of rheumatoid arthritis. *Nat Med J China* 2000; 80: 20-4.
21. WANG Y, SHI J, CHEN W: The diagnosis significance of anti-RA33 and anti-CCP in rheumatoid arthritis. *Chongqing Medical* 2003; 32: 1033.
22. CUI T, XIE J, YU L, HU L, SHEN L, WU J: The application of anti-CCP, anti-RA-33 and other four autoantibodies in the diagnosis of rheumatoid arthritis. *Shanghai J of Med Lab Sci* 2003; 18: 221.
23. YANG L, YU M, LIN J, GAO Y, YIN H: Detecting anti-heterogeneous nuclear ribonucleoprotein A2/RA33 antibody by enzyme-linked immunosorbent assay. *Chin J Lab Med* 2003; 26: 428-30.
24. ZHONG L-Y, LI R-Y, ZENG Z-H: The diagnostic value of anti-cyclic citrullinated peptide antibody and anti-RA33 antibody performed concomitantly with RF in patients with rheumatoid arthritis. *Proc Clin Med J* 2004; 13: 568.
25. ZHANG H, YE Z, LIU G, XU Z, LIU Q: The combined detection significance of RF, anti RA33 antibody, anti-CCP antibody in the diagnosis of rheumatoid arthritis. *Guizhou Medical Journal* 2004; 28.
26. MEI X, LIU Y, ZHOU Y: The clinical significance of detecting serum auto-antibodies in early diagnosis of Rheumatoid arthritis. *Jiangxi J Med Lab Sci* 2004; 22: 395.
27. CHENG P, LIU Y, CHEN S: Clinical evaluation of early marker in diagnosing rheumatoid arthritis. *Medical Journal of Wuhan University* 2005; 26: 529.
28. LIU X, YU X,YU Y: The use of one-step immunoblotting to detect both anti-RA33 and anti) ENA antibodies, and its diagnostic value for RA patients. *Chin J Lab Diagn* 2005; 9: 754.
29. CHENG Y, WANG X, FAN C: Application of measurements of serum anti-CCP and anti-RA33in diagnosis of early rheumatoid arthritis. *J Postgrad Med* 2005; 28: 22.
30. LEI X, LI S: Diagnosis value of anti-CCP, anti-RA33 and RF in rheumatoid arthritis *J Clin Intern Med* 2005; 22: 667.
31. CHEN L, LI Z, FAN X, DUAN J: Differential diagnosis value and one year follow-up study of anti-RA33 in patient of rheumatoid arthritis. *J Pract Med* 2005; 21: 1141.
32. NELL VP, MACHOLD KP, STAMM TA, EBERL G *et al*.: Autoantibody profiling as early diagnostic and prognostic tool for rheumatoid

arthritis. *Ann Rheum Dis* 2005; 64: 1731-6.
33. LI S, MA J, WANG Y, WANG Y, DONG A: The clinical value of anti CCP antibody, anti RA33 antibody and CRP in rheumatoid arthritis patients. *Chinese Journal of Clinical Laboratory Science* 2006; 24:270.
34. CHEN M, LI H, NING Q: The clinic significance of combined detection anti-CCP, anti-AKA and anti-RA33 in the early diagnosis rheumatoid arthritis. *Modern Hospital* 2006; 6: 60
35. GUO Y, CHEN Z, GU M: Diagnostic value of citrullinated protein antibody and anti-RA33 antibody in patients with rheumatoid arthritis. *Jiangsu Med J* 2006; 32: 112.
36. GU F, ZHANG Y, SHI J: The diagnostic significance of combined detection of anti-CCP antibody, anti-RA33 antibodies, anti-Sa antibody and RF in rheumatoid arthritis. J*iangxi J. Med Lab Sci* 2007; 25: 24.
37. DAI L-Q, WY-Q, ZHANG L, LI Q-W, SUN X-Z: The diagnostic significance of anti cyclic citrullinated peptide antibody and antiRA33antibody in rheumatoid arthritis. *Acta Acad Med Weifang* 2007; 29: 298.
38. WANG L, GU M: The significance of combined detection of anti CPA, RA33 and RF in rheumatoid arthritis. *Journal of Clinical Medicine in Practice* 2007; 11: 110.
39. DUAN F-L, LI Y, CHENG G-J, HU X-M: Diagnostic value of anti-CCP antibody, anti-AKA antibody and anti-RA33antibody in patients with rheumatoid arthritis. *J Mod Lab Med* 2008; 23: 94.
40. WANG L, HAN B, WANG H: The clinical significance of detecting anti-CCP and anti-RA33 antibodies for rheumatoid arthritis patients. *Chinese Joumal of Medicinal Guide* 2008; 10.
41. CHUNMEI J: Use of anti-cyclic citrullinated peptide and anti-RA33antibodies in diagnosing rheumatoid arthritis. *Medical Laboratory Science and Clinics* 2008; 19: 84.
42. WANG C, HU X, ZHANG H: The value of serum anti CCP, anti RA33 antibodies in the diagnosis of rheumatoid arthritis. *Shandong Medical J* 2008; 48: 84.
43. GUIXIANG Z: The detection analysis of three kinds of antibodies in serum of patients with rheumatoid arthritis. *Chin J Misdiagn* 2008; 8: 5578.
44. ZENG H-Q, ZD.-X, YU H-G: Clinical value of combined tests of three antibodies assaies in the diagnosis of early stage for rheumatoid arthritis. *Chinese General Practice* 2008; 11: 650.
45. YU Y, Z.X.-N., CHENG X-D, HAO X-K, PENG D-R: Clinical significance of combined detection of RF, Anti-RA33 antibody, CRP, ANA with ENA in diagnosis, treatment and predicting procession of rheumatoid arthritis. *Progress in Modern Biomedicine* 2009; 9: 3104.
46. ZHANG W, ZHENG J, ZHAO Y: The diagnostic value of anti CCP and anti RA33 antibody in the diagnosis of rheumatoid arthritis. *Shaanxi Medical J* 2009; 38: 716.
47. GUO X-H, PAN Y-F, WEI Q-J: Diagnostic role of anti-heterogeneous nuclear ribonucleoprotein A2 antibody in rheumatism. *Chinese Journal of General Practice* 2009; 7: 795.
48. HE Z: Detection and clinical evaluation of autoantibodies in patients with rheumatoid arthritis. *Guangzhou Medical University* 2010.
49. OUYANG Y, JIANG Y, TAN L *et al*.: The clinical significance of detecting HLA-DR and autoantibodies in patients with rheumatoid arthritis. *Shandong Medical J* 2010; 50: 77.
50. ZHANG G, CHANG L, FANG X: The diagnostic significance of anti CCP antibody combined with anti RA33 antibody and RF in the diagnosis of rheumatoid arthritis. *Chin J Clin Lab Sci* 2010; 28: 196.
51. WANG Y, DING Y, MA J: Clinical value of anti RA33, anti CCP antibody and RF in the diagnosis of RA. *Medical Journal of Chinese People's Health* 2010; 22: 2084.
52. LE H: Preliminary study and application of anti CCP combined with anti RA33 antibody and RF in the diagnosis of rheumatoid arthritis. *Contemporary Medicine* 2011; 17: 145.
53. ZHU H, TONG T: The diagnostic value of anti CCP antibody, RA33 antibody and IgG-Rf factor in rheumatoid arthritis. *Traditional Chinese Medicine in Inner Mongolia* 2011; 11: 111.
54. QIN W, DENG Y, XU P: Application of RF, anti RA33 and anti CCP antibody in diagnosis of rheumatoid arthritis. *Laboratory Medicine* 2011; 26: 703.
55. ZHANG Y: The explore application of laboratory diagnosis of rheumatoid arthritis. *China Prac Med* 2011; 6: 113.
56. ZHANG W, HU T, WANG Y: The significance of multiple autoantibodies detection in the early diagnosis of rheumatoid arthritis. *Chinese Remedies and clinics* 2011; 11: 965.
57. YAO Y, PENG Q, LI S: Diagnostic value of three auto-antibodies in patients with rheumatoid arthritis. *Lab Med Clin* 2012; 9: 2840.
58. CHEN H, ZENG M: Joint detection of anti-MCV, anti-RA33 antibody, RF in diagnosis of rheumatoid arthritis. *Chinese Journal of Health Laboratory Technology* 2013; 23: 399.
59. CHEN C, HE L, CAO Q, ZHAO L: Clinical significance on antiperinuclear factor, antikeratin antibody, anti-RA33 and anti-cyclic citrullinated peptide antibody in the diagnosis of rheumatoid arthritis. *Int J Lab Med* 2013; 34: 769.
60. NIU R, GUO L: Clinical application evaluation of several autoantibodies in the diagnosis of rheumatoid arthritis. *Laboratory Medicine* 2013; 28: 657.
61. MOHAMMED MARROF AL-ANI. MSC, F: Comparison between Anti-filaggrin, Anti-RA33 and Anti-Cyclic Citrullinated Peptide Antibodies in the Diagnosis of Rheumatoid Arthritis in Iraqi Patients. *Iraqi J Comm Med* 2012; 4: 337.
62. YONGMEI ZHOU LT, QIUHUA Q, HUA L *et al*.: Study of association between HLA-DR4 and DR53 and autoantibody detection in rheumatoid arthritis *Journal of Immunoassay and Immunochemistry* 2013; 34: 126-33.
63. ZHONG RY, MINNAN L, LIYUN LS: The value of combined detection of serum RF, anti-RA33, Anti-CCP and ESR in the diagnosis of early rheumatoid arthritis. *China and Foreign Medical Treatment* 2014; 3: 3.
64. CHEN S, LV C, LI D: Diagnostic value of selective combining detection with anti-CCP, anti-MCV, RF, and anti-RA33 in rheumatoid arthritis. *Journal of Jilin Medical college* 2014; 35: 7.
65. ZHENG H-X: The clinical value of combined detection of varied serum index variety serological markers in the diagnosis of rheumatoid arthritis. *China Modern Medicine* 2014; 21: 86.
66. GAO L: The clinical diagnostic value of anti -CCP, anti-RA33 antibody combined with RF in the diagnosis of rheumatoid arthritis. *J Shanxi Med Univ* 2014; 45: 116.
67. LASHKARI M, NOORI A, HAJIIMAN-OUCHEHRI F, OVEISI S, KAZEMIFAR AM: Determination of specificity and sensitivity of anti-RA 33 in diagnosis of early rheumatoid arthritis. *Glob J Health Sci* 2014; 6: 292-7.
68. AL-MUGHALES JA: Immunodiagnostic significance of anti-RA33 autoantibodies in Saudi patients with rheumatoid arthritis. *J Immunol Res* 2015; 2015: 604305.
69. WANG T, HE Y: Diagnostic value of combined detection of AKA antibody, anti CCP antibody, anti RA33 antibody and RF for rheumatoid arthritis. *China Modern Doctor* 2015; 53: 96.
70. ZAMORA J, ABRAIRA V, MURIEL A, KHAN K, COOMARASAMY A: Meta-DiSc: a software for meta-analysis of test accuracy data. *BMC Medical Research Methodology* 2006; 6: 31.
71. NISHIMURA K, SUGIYAMA D, KOGATA Y *et al*.: Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. *Ann Intern Med* 2007; 146: 797-808.
72. SUN J, ZHANG Y, LIU L, LIU G: Diagnostic accuracy of combined tests of anti cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis: a meta-analysis. *Clin Exp Rheumatol* 2014; 32: 11-21.
73. MEYER O, LABARRE C, DOUGADOS M *et al*.: Anticitrullinated protein/peptide antibody assays in early rheumatoid arthritis for predicting five year radiographic damage. *Ann Rheum Dis* 2003; 62: 120-6.
74. VENCOVSKY J, MACHACEK S, SEDOVA L *et al*.: Autoantibodies can be prognostic markers of an erosive disease in early rheumatoid arthritis. *Ann Rheum Dis* 2003; 62: 427-30.
75. TRONSTROM J, DRABORG AH, HANSEN PR, HOUEN G, TRIER NH: Identification of a linear epitope recognized by a monoclonal antibody directed to the heterogeneous nucleoriboprotein A2. *Protein Pept Lett* 2014; 21: 25-31.
76. RONNELID J, WICK MC, LAMPA J *et al*.: Longitudinal analysis of citrullinated protein/peptide antibodies (anti-CP) during 5 year follow up in early rheumatoid arthritis: anti-CP status predicts worse disease activity and greater radiological progression. *Ann Rheum Dis* 2005; 64: 1744-9.
77. GLAS AS, LIJMER JEROEN G, PRINS MH *et al*.: The diagnostic odds ratio: a single indicator of test performance. *J Clin Epidemiol* 2003; 56: 1129-35.
78. JONES CM, ATHANASIOU T: Summary receiver operating characteristic curve analysis techniques in the evaluation of diagnostic tests. *Ann Thorac Surg* 2005; 79: 16-20.
79. WALTER SD: Properties of the summary receiver operating characteristic (SROC) curve for diagnostic test data. *Stat Med* 2002; 21: 1237-56.