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A Retrospective Study: The Significance of Combined Testing of Serum Markers for Diagnosis of Rheumatoid Arthritis

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Background: There have been few studies on the value of various antibody combinations in rheumatoid arthritis (RA) diagnosis, and a lack of studies with large sample sizes, especially in the Chinese population. This study retrospectively evaluated the diagnostic value of a combined assay of five auto-antibodies [anti-cyclic citrullinated peptide (anti-CCP), anti-keratin (AKA), anti-RA 33, glucose-6-phosphate isomerase (GPI), and rheumatoid factor (RF)] for RA.

Material/Methods: Data were obtained from 5,725 patients with rheumatic diseases in Southwest Hospital of Chongqing from 2011 to 2014. Detection of the five serological markers was performed for all study patients using the appropriate method for each antibody.

Results: It was found that of the 5,725 patients, the positive rates for RF, anti-CCP, anti-RA 33, AKA, and GPI were 52.5%, 40.1%, 12.8%, 12.0%, and 50.0% respectively. In RA patients, the positive rates were 83.3%, 68.5%, 16.6%, 20.8%, and 77.9% respectively, which were all significantly higher than those detected in patients with the other diseases ($p<0.01$). The areas under the receiver operator characteristic (ROC) curve for RF, anti-CCP, anti-RA 33, AKA, and GPI were 0.857, 0.831, 0.528, 0.602, and 0.822 respectively, indicating that these five serological markers display favorable diagnostic value for RA. There were positive correlations between anti-CCP antibody and RF and GPI ($p<0.01$) and between RF and GPI ($p<0.01$), but no correlation between anti-RA 33 and AKA ($p<0.01$). The specificity of the combination of anti-CCP, AKA, and GPI was 100% for RA diagnosis.

Conclusions: The combined assay of serological markers significantly improved the diagnostic specificity for RA. The diagnostic value of RF for RA was the highest and the combined assay for anti-CCP, AKA, and GPI had the highest specificity for RA diagnosis.

MeSH Keywords: Arthritis, Rheumatoid • Biological Markers • Rheumatoid Factor • Sensitivity and Specificity

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Background

Rheumatoid arthritis (RA) is a common, systemic, autoimmune disease of unknown etiology that is characterized by chronic erosive arthritis. Irreversible bone destruction can be found in RA patients two years after onset [1]. As RA is an autoimmune disease, there are many auto-antibodies produced in the serum of patients during disease progression. Hence, a specific and sensitive serological test is needed for early diagnosis and early targeted intensive therapy to support achieving good disease control [1]. Many serum markers of RA have been identified, such as rheumatoid factor (RF), anti-CCP antibody, anti-keratin antibody (AKA), glucose-6-phosphate isomerase (GPI), anti-RA 33 antibody, anti-Sa antibody, anti-perinuclear factor antibody, and anti-mutated citrullinated vimentin (MCV) antibody [2–5]. The value of these markers in RA diagnosis has been reported, however, the results are variable. It is widely accepted that RF, anti-CCP antibody, anti-RA 33 antibody, AKA, and GPI are sensitivity and specificity for RA diagnosis [6,7]. Recently, it was found that anti-Ig heavy chain binding protein and CCP antibodies detected in tandem combination can obtain higher specificity and have good clinical value for the differential diagnosis of RA [6,7]. However, there have been few studies on the value of various antibody combinations in RA diagnosis and a lack of studies with large sample sizes, especially in the Chinese population [2–5].

In this study, we assessed the values of RF, anti-CCP antibody, anti-RA 33 antibody, AKA, and GPI alone and in combination in RA diagnosis. We screened serum markers and their combinations with relatively high diagnostic values for RA. The clinical data, diagnosis, and test results of 5,725 patients (both inpatients and outpatients) who visited Southwest Hospital of Chongqing between January 2011 and December 2014 and underwent testing for the five serum markers were analyzed retrospectively to determine the diagnostic value of these markers for RA. Furthermore, the specificity and sensitivity of combined testing of various markers were analyzed for RA diagnosis.

Material and Methods

Study patients

The study included 5,725 patients (both inpatients and outpatients) with rheumatic diseases (aged 5–75 years (mean ± standard deviation (SD); 40.6±17.3 years); 1,444 males; 4,281 females) who visited our hospital between January 2011 and December 2014. These included study patients were diagnosed with the following diseases: 3,342 with RA, 1,446 with osteoarthritis (OA), 209 with systemic lupus erythematosus (SLE), 264 with ankylosing spondylitis (AS), 63 with mixed connective tissue disease (MCTD), 133 with undifferentiated connective tissue disease (UCTD), 60 with Sjogren syndrome (SS), 47 with polymyositis/dermatomyositis (PM/DM), 45 with systemic sclerosis (SSc), 39 with juvenile idiopathic arthritis (JIA), 38 with psoriatic arthritis (PsA), 29 with gout arthritis (GA), and 10 with Behçet disease (BD). The diagnosis of these diseases was made according to the American College of Rheumatology (ACR) criteria or other diagnostic criteria. The diagnosis of RA was based on ACR/EULAR (European League against Rheumatism) 2010 rheumatoid arthritis classification criteria [8]. Patient data are shown in Table 1.

Inclusion and exclusion criteria

All patients with rheumatic diseases that were included in this study had a diagnosis on the first visit to hospital and were tested for the five serological indexes at the same time. The following exclusion criteria applied to all participants: unclear diagnosis and overlap with other connective tissue diseases.

Patient data analysis

The study was approved by the Southwest Hospital Ethical Committee in 2010. As a retrospective study, patient consents were not obtained. The patient records/information was anonymized and de-identified prior to analysis. The data of inpatients and outpatients who underwent testing of the five serum markers and had a definite diagnosis and complete medical records were analyzed statistically.

Reagents and testing methods

Each of the serological indexes was measure as follows. For RF, we used nephelometry (reagents and standards provided by Beckman, USA); positive result if ≥20 U/mL. For GPI, we used double antibody sandwich ELISA (kit provided by Shanghai Beijia Biochemical Reagents, China); positive result if ≥0. 209 mg/L. For AKA, we used indirect immunofluorescence assay (reagents provided by Euroimmun Medizinische Labordiagnostika AG, Germany); positive result if ≥1: 10. For anti-RA 33 antibody, we used ELISA (kit provided by Shenzhen YHLO Biotech, China); positive result if ≥25 RU/mL. For anti-CCP antibody, we used ELISA (kit provided by Shanghai Fuchun Reagents Co., China; positive result if ≥25 RU/mL. All kits were used according to manufacturers' instructions.

Statistical analysis

Statistical analysis was performed using SPSS17.0 software. Crosstabs were used to calculate the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), negative likelihood ratio (NLR) of one, two or more serum markers for RA diagnosis. Enumeration data were subject to chi-square test. A value of $p<0.05$ was

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Table 1. Clinical data of patients.

Group	No. of patients	Male (n)	Female (n)	M/F	Age range (y)	Mean age ±SD (y)
RA	3,342	807	2,535	1: 3.1	16–75	43±13.1
OA	1,446	290	1156	1: 3.9	38–75	66.2±7
AS	264	214	50	4.3: 1	16–72	35.3±13.1
SLE	209	5	204	1: 40.8	12–62	39.3±13.1
UCTD	133	16	117	1: 7.3	14–69	45±13
MCTD	63	5	58	1: 11.6	19–64	41.3±11.7
SS	60	3	57	1: 19	18–74	53.1±11.1
PM/DM	47	21	26	1: 2	16–52	39.6±13.2
SSc	45	9	36	1: 7.2	24–64	47.6±12.3
JIA	39	22	17	1.3: 1	5–15	10.8±3.9
PsA	38	20	18	1: 0.9	27–75	51.7±12.2
GA	29	29			13–73	46.1±13.4
BD	10	3	7	1: 2.3	21–41	35.6±16.6

RA – rheumatoid arthritis; OA – osteoarthritis; AS – ankylosing spondylitis; SLE – systemic lupus erythematosus; UCTD – undifferentiated connective tissue disease; MCTD – mixed connective tissue disease; SS – Sjögren's syndrome; PM/DM – polymyositis/dermatomyositis; SSc – systemic sclerosis, JIA – juvenile idiopathic arthritis, PsA – psoriatic arthritis; GA – gout arthritis; BD – Behçet disease.

considered to indicate statistical significance. Pearson linear correlation analysis and kappa testing were also performed.

Results

Overall positive rates of the five serum markers

Of the 5,725 patients with rheumatic diseases, the positive rates of RF, anti-CCP antibody, anti-RA 33 antibody, AKA, and GPI were 52.5%, 40.1%, 12.8%, 12.0%, 50.0% respectively. The GPI-positive rate was the highest, and the AKA positive rate was the lowest.

Positive rates of the five serum markers in the various disease groups

The positive rates of RF, anti-CCP antibody, AKA, and GPI were significantly higher in RA patients than in the other groups ($p<0.01$). There were no significant differences in the positive rates of anti-RA 33 antibody among the RA, MCTD, UCTD, and SSc patients ($p>0.05$), while the positive rate of anti-RA 33 antibody was markedly higher in the RA group than in the other disease groups ($p<0.01$). Table 2 shows the positive rates of the five serum markers in the various disease groups.

Diagnostic value of the five serum markers for RA

The diagnostic specificity, sensitivity, PPV, NPV, PLR, NLR, and Youden index (YI) of RF, anti-CCP antibody, anti-RA 33 antibody, AKA, and GPI for RA are presented in Table 3. As shown, the sensitivity of RF was the highest (83.3%) and the specificity of AKA was the highest (99.6%), the PPV of AKA was the highest (98.6%), the NPV of RF was the highest (75.5%), the PLR of AKA was the highest (47.7), the NLR of RF was the lowest (0.2), and the YI of RF was the highest (0.7). Figure 1 shows the receiver operator characteristic (ROC) curves of the five serum markers for RA diagnosis. The areas under the ROC curves of RF, anti-CCP antibody, anti-RA 33 antibodies, AKA, and GPI for RA diagnosis were 0.857, 0.831, 0.528, 0.602, and 0.822 respectively. The diagnostic value of RF for RA was the highest.

Relationships between the five serum markers

The relationships between the five serum markers are shown in Table 4. There was a certain consistency between anti-CCP antibody and the RF-positive rate ($\kappa=0.285$, $P<0.01$). Anti-CCP antibody partly consistented with the GPI-positive rate ($\kappa=0.291$, $P<0.01$). RF consistented with the GPI-positive rate ($\kappa=0.345$, $P<0.01$). Anti-RA 33 antibody did not consistent with the AKA positive rate ($\kappa=0.000$, $P<0.01$).

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Table 2. Positive rates of the five serum markers in the various disease groups [n (%)].

Group	No. of patients	RF		Anti-CCP antibody		Anti-RA 33 antibody		AKA		GPI	
RA	3,342	2,784	(83.3%)*	2,290	(68.5%)*	556	(16.6%)	694	(20.8%)*	2,602	(77.9%)*
OA	1446	82	(5.7%)	4	(0.3%)	13	(0.9%)*	1	(0.1%)	38	(2.6%)
AS	264	15	(5.7%)	3	(1.1%)	19	(7.2%)*	2	(0.8%)	80	(30.3%)
SLE	209	47	(22.5%)	8	(3.8%)	77	(36.8%)#	0	(0%)	73	(34.9%)
UCTD	133	37	(27.8%)	12	(9%)	32	(24.1%)#	1	(0.8%)	32	(24.1%)
MCTD	63	44	(69.8%)	7	(11.1%)	33	(52.4%)#	1	(1.6%)	22	(34.9%)
SS	60	34	(56.7%)	4	(6.7%)	3	(5%)*	1	(1.7%)	24	(40%)
PM/DM	47	5	(10.6%)	2	(4.3%)	4	(8.5%)*	2	(4.3%)	14	(29.8%)
SSc	45	9	(20.8%)	4	(8.9%)	5	(11.1%)*	0	(0%)	13	(28.9%)
JIA	39	4	(10.3%)	4	(10.3%)	2	(5.1%)*	0	(0%)	16	(41%)
PsA	38	7	(18.4%)	6	(15.8%)	1	(2.6%)*	2	(5.3%)	8	(21.1%)
GA	29	2	(6.9%)	0	(0%)	1	(3.4%)*	0	(0%)	2	(6.9%)
BD	10	0	(0%)	0	(0%)	1	(10%)*	0	(0%)	1	(10%)

* $p<0.01$, # $p>0.05$, RA group versus the other disease groups. RA – rheumatoid arthritis; OA – osteoarthritis; AS – ankylosing spondylitis; SLE – systemic lupus erythematosus; UCTD – undifferentiated connective tissue disease; MCTD – mixed connective tissue disease; SS – Sjögren's syndrome; PM/DM – polymyositis/dermatomyositis; SSc – systemic sclerosis, JIA – juvenile idiopathic arthritis, PsA – psoriatic arthritis; GA – gout arthritis; BD – Behçet disease; anti-CCP – anti-cyclic citrullinated peptide; AKA – anti-keratin; GPI – glucose-6-phosphate isomerase; RF – rheumatoid factor.

Sensitivity and specificity of the five serologic markers in combination

Table 5 shows the number of positive patients, and sensitivity and specificity of the assays of the five markers in various combinations. Of the combinations of two markers, the sensitivity of GP + RF was the highest (67.8%), and the specificity of anti-CCP antibody + AKA and anti-CCP antibody + anti-RA 33 antibody was the highest (99.9%). Of the combinations of three markers, the sensitivity of anti-CCP antibody + GPI + RF was the highest (53.3%), and the specificity of anti-CCP antibody +AKA + GPI was the highest (100%). Of the combinations of four markers, the sensitivity of anti-CCP antibody + AKA + GPI + RF was the highest (15.3%), and the specificity of that combination was also the highest (100%). The combination of all five markers exhibited a sensitivity of 2.0% and a specificity of 100%.

Discussion

Currently, the 2010 ACR/EULAR Classification Criteria for rheumatoid arthritis are widely accepted [8]. In contrast, according to the 1987 ACR Classification Criteria, RA diagnosis is based primarily on clinical presentation, radiologic changes, and rheumatoid factor test results [9]. At the point when all the criteria are met, the patient usually has bone destruction and irreversible joint damage. In addition, due to inadequate specificity, RF testing does not aid early diagnosis and treatment [10]. The 2010 ACR/EULAR Classification Criteria, which are not as strict as the 1987 criteria, describe the diagnostic value of serologic markers while excluding radiologic examinations [11]. High-titer RF or auto-antibodies against citrullinated proteins (ACPA) score three points; hence, patients easily accumulate six points, thus aiding early diagnosis of RA. On the other hand, the likelihood of misdiagnosis may increase [10]. In addition to RA, many rheumatic diseases sometimes present with arthritis at onset or later [9,12]. Therefore, besides clinical presentation, medical history and imaging examinations, highly sensitive and specific serologic tests may be utilized to distinguish RA from other disorders. The value of RF, anti-CCP antibody, anti-RA 33 antibody, AKA, GPI, and other serum markers in RA diagnosis has been reported in a number of studies [7]. However, the combinations of RF, anti-CCP antibody, anti-RA 33 antibody, AKA, and GPI have seldom been reported and were based on small sample sizes with highly variable sensitivity, specificity, PPV, NPV, PLR, and NLR for the various serum markers. In this study, the ideal control population was

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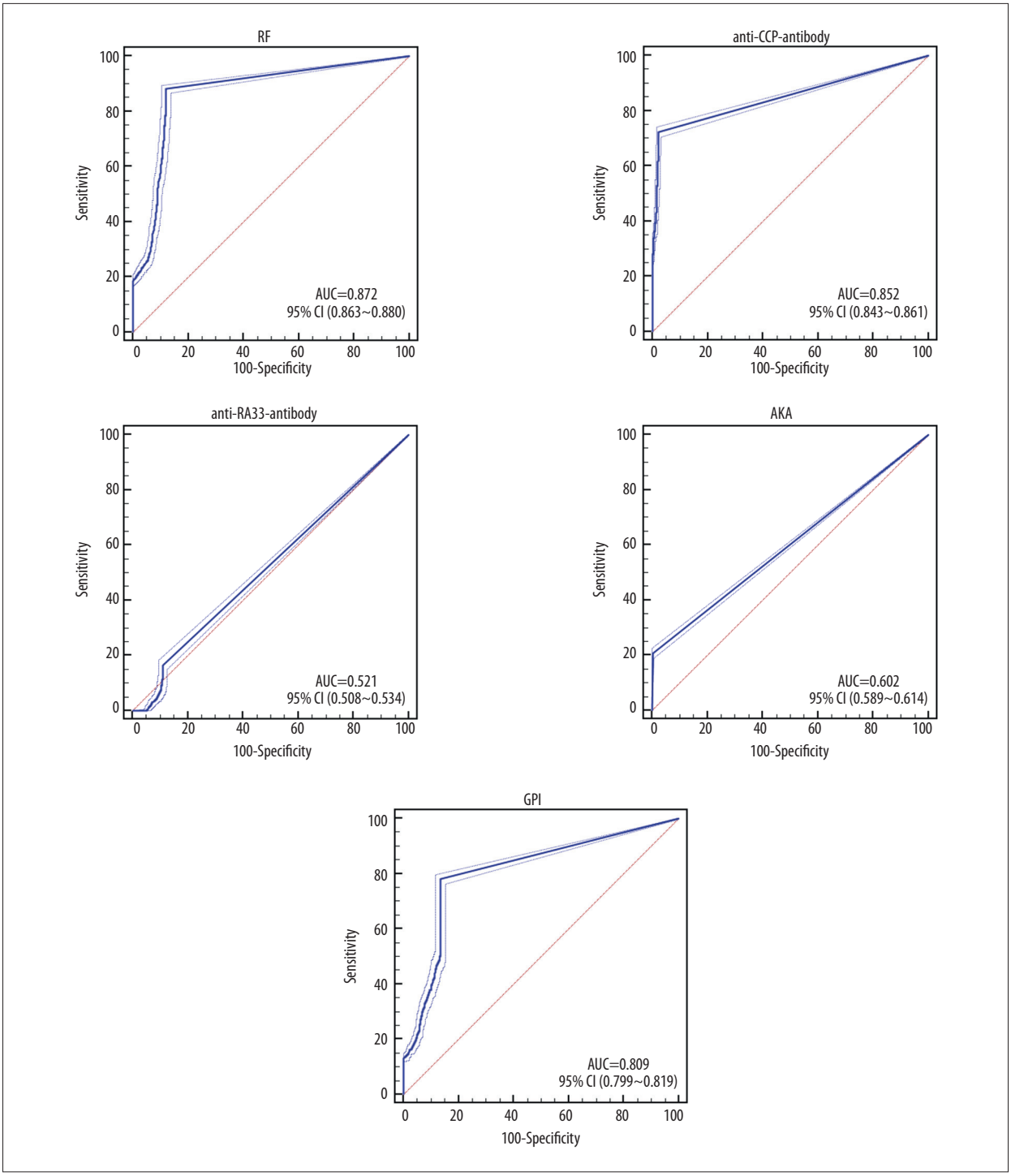

Figure 1. Receiver operator characteristic (ROC) curves of the five serologic markers for RA diagnosis

believed to be patients who may be prescribed with the tests for the five serological markers. Hence, the study participants were those patients who visited our hospital and presented with arthritis. We did not enroll healthy people alone in the control group, because this would increase the specificity of tests. This retrospective study assessed sensitivity, specificity, PPV, NPV, PLR, and NLR of five serum markers and their various combinations to identify the serum markers and their combinations with high diagnostic value for RA and provide support for the early diagnosis of RA.

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Table 3. Diagnostic value of the five serum markers for RA.

Parameters	Se (%)	Sp (%)	PPV (%)	NPV (%)	PLR	NLR	YI
RF	83.3%	88.0%	90.7%	75.5%	6.7	0.2	0.7
Anti-CCP antibody	68.5%	97.7%	97.7%	66.5%	29.2	0.3	0.6
Anti-RA 33 antibody	16.6%	92.0%	74.4%	43.0%	2.0	0.9	0.1
AKA	20.0%	99.6%	98.6%	46.1%	47.7	0.8	0.2
GPI	77.9%	86.4%	89.0%	70.5%	5.5	0.3	0.6

Se – sensitivity; Sp – specificity; PPV – positive predictive value; NPV – negative predictive value; PLR – positive likelihood ratio; NLR – negative likelihood ratio; YI – Youden index; anti-CCP – anti-cyclic citrullinated peptide; AKA – anti-keratin; GPI – glucose-6-phosphate isomerase; RF – rheumatoid factor.

Table 4. Relationships between the five serum markers.

		Anti-RA33 antibody		AKA		GPI		Anti-CCP antibody	
		+	–	+	–	+	–	+	–
RF	+	478	2306	641	2143	2349	435	2087	697
	–	78	480	53	505	253	305	203	355
		κ=0.012	p=0.000	κ=0.054	p=0.000	κ=0.345	p=0.000	κ=0.285	p=0.000
Anti-ccp antibody	+	412	1878	607	1683	1976	314		
	–	144	908	87	965	626	426		
		κ=0.030	p=0.000	κ=0.129	p=0.000	κ=0.291	p=0.000		
GPI	+	459	2143	616	1986				
	–	97	643	78	662				
		κ=0.023	p=0.000	κ=0.068	p=0.000				
AKA	+	88	606						
	–	468	2180						
		κ=0.000	p=0.000						

κ – results of kappa testing. anti-CCP – anti-cyclic citrullinated peptide; AKA – anti-keratin; GPI – glucose-6-phosphate isomerase; RF – rheumatoid factor.

In this study, the sensitivity of RF, anti-CCP antibody, anti-RA 33 antibody, AKA, and GPI was 83.3%, 68.5%, 16.6%, 20.8%, and 77.9% respectively; the specificity was 88.0%, 97.7%, 92.0%, 99.6%, and 86.4% respectively. In studies by Liu et al. [13], Nikbin et al. [14], Lin et al. [15], Goeldner et al. [16], and Zhu et al. [17], the sensitivity of RF ranged from 56.2% to 81.5%, and the specificity ranged from 61.3% to 83.5% in RA patients. In studies by Liu et al. [13], Lin et al. [15], Debaugnies et al. [18], and Shidara et al. [19], the sensitivity of anti-CCP antibody ranged from 51.2% to 76.5%, and the specificity ranged from 89.9% to 99.0% in RA patients. In studies by Lin et al. [15], Zhu et al. [17], Fusconi et al. [20], and Cordonnier et al. [21], the AKA positive rate ranged from 36.2% to 48.7%, and its specificity ranged from 89.9% to 98.0% in RA patients. In studies by Cordonnier et al. [21], Maslyanskiy et al. [22], Lashkari et al. [23] and Mediwake et al. [24], the sensitivity of anti-RA 33 antibody ranged from 28.9% to 44.7%, and the specificity ranged from 89.9% to 95.0% in RA patients. In studies by Zhu et al. [17], Fan et al. [25], Chen et al. [26] and Dai et al. [27], the sensitivity of GPI ranged from 33% to 81.6%, and the specificity ranged from 55.7% to 91.5% in RA patients. Our results for RF, anti-CCP antibody, and GPI are consistent with these reports, while the sensitivity of anti-RA 33 antibody and AKA were significantly lower than those previously reported. These discrepancies may relate to the composition of the control and RA patient groups, and the definition of positive values. In addition, AKA was tested by indirect immunofluorescence assay, the results of which are open to subjective interpretation; therefore, the accuracy of results will be influenced by the experience of the examiner. Multiple center studies with large sample sizes are required to analyze the sensitivity and specificity of anti-RA 33 antibody and AKA for the diagnosis of RA.

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Table 5. Sensitivity and specificity of the five serologic markers in combination.

Combination	No. of patients (+)		Sensitivity	Specificity	Combination	No. of patients (+)		Sensitivity	Specificity
	RA	Non-RA				RA	Non-RA		
CCP+AKA	607	3	17.5%	99.9%	CCP+AKA+RA 33	79	2	2.3%	99.9%
CCP+GPI	1976	25	57.0%	99.0%	CCP+GPI+RA 33	361	4	10.4%	99.8%
CCP+RA 33	412	3	11.9%	99.9%	CCP+RA 33+RF	366	6	10.6%	99.7%
CCP+RF	2087	4	60.2%	99.8%	AKA+GPI+RA 33	81	7	2.3%	99.7%
AKA+GPI	616	5	17.8%	99.8%	AKA+GPI+RF	579	8	16.7%	99.7%
AKA+RA 33	88	6	2.5%	99.7%	AKA+RA 33+RF	78	9	2.3%	99.6%
AKA+RF	641	7	18.5%	99.7%	GPI+RA 33+RF	407	10	11.7%	99.6%
GPI+RA 33	459	8	13.3%	99.7%	CCP+GPI+RF	1845	5	53.3%	99.8%
GPI+RF	2349	9	67.8%	99.6%	AKA+GPI+RA 33+RF	72	3	2.1%	99.9%
RA 33+RF	478	10	13.8%	99.6%	CCP+GPI+RA 33+RF	327	4	9.4%	99.8%
CCP+AKA+GPI	556	1	16.6%	100.0%	CCP+AKA+RA33+RF	74	5	2.1%	99.8%
CCP+AKA+RF	571	3	16.5%	99.9%	CCP+AKA+GPI+RF	529	1	15.3%	100%
CCP+AKA+GPI RA 33	74	1	2.1%	100%	CCP+AKA+GPI RA33+RF	69	1	2.0%	100.0%

CCP – anti-CCP antibody; RA 33 – anti-RA 33 antibody; AKA – anti-keratin; GPI – glucose-6-phosphate isomerase; RF – rheumatoid factor; RA – rheumatoid arthritis.

We found that the sensitivity of RF was similar to that of GPI, but was markedly higher than that of anti-CCP antibody, anti-RA 33 antibody, and AKA. However, the specificity of RF and GPI was markedly lower than that of anti-CCP antibody and AKA. We also found that the PPV of anti-CCP was markedly higher than that of GPI and RF, and the NPV of RF was similar to that of GPI, but was markedly higher than that of anti-RA 33 antibody and AKA. The PLR of anti-CCP antibody was markedly higher than that of GPI and RF, and the NLR of anti-CCP antibody was similar to that of GPI and RF, but markedly lower than that of anti-RA33 antibody and AKA. The YI and area under ROC curve of anti-CCP antibody, RF, and GPI were significantly higher than those of anti-RA 33 antibody, and AKA. The sensitivity of RF was the highest; hence, RF should be the preferred marker for large-scale population screening for RA. The specificity of AKA was the highest. However, AKA was tested by indirect immunofluorescence assay, which is subject to numerous confounding factors, including human bias, making quality control and standardization difficult. Anti-CCP antibody was tested by ELISA. This method is easy to perform, and can be standardized and quality controlled; therefore, if RA is suspected, anti-CCP antibody can be used as a confirmatory parameter. The sensitivity of GPI was lower than that of RF, but higher than that of anti-CCP antibody; hence, GPI can be used as a backup parameter for discriminative diagnosis. Therefore, combined testing for RF, anti-CCP, and GPI should be performed early in patients with typical clinical presentations and a strong suspicion of RA.

The correlations among these five specific serologic markers suggest that they supplement each other in RA diagnosis. RA patients negative for one marker may be positive for any of the other four markers, and anti-CCP antibody correlated positively with RF and GPI positive rates. The RF-positive rate correlated positively with the GPI positive rate. The anti-RA 33 antibody positive rates correlated weakly with the RF positive rate. The anti-RA 33 antibody positive rates did not correlate to the AKA positive rate. These findings were consistent with other studies [5,17]. In RA diagnosis, anti-RA 33 antibody did not correlate with AKA, and correlated weakly with RF and anti-CCP antibody. In anti-CCP antibody, AKA or RF-negative patients, anti-RA 33 antibody may be positive. Hence, anti-RA 33 antibody is a highly RA specific antibody, and helps prevent missed diagnosis of RA.

In the present study, combined testing of the fives serological markers increased testing specificity substantially, while the sensitivity of the various combinations of multiple markers

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tended to decrease as the number of markers in the combination increased. It can be speculated that this effect relates to poor coincidence of the combined examinations, and is similar to the findings of Bas et al. [28] and Zhang et al. [29]. Therefore, when the diagnostic significance of serum markers in combination is evaluated, sensitivity and specificity, PPV and NPV, PLR and NLR, as well as cost-effectiveness should be considered, so as to maximize the value of such combinations. RF testing is economical and preferred for large-scale population screening. RF testing methods include the latex agglutination assay, immune-turbidimetric assay, and ELISA; the latter two methods require dedicated equipment and laboratory technicians. Anti-CCP antibody can be tested by ELISA and colloidal gold immune-chromatographic assay. The latex agglutination assay for RF and colloidal gold immune-chromatographic assay for anti-CCP antibody do not require any dedicated equipment, and the testing procedure lasts only several minutes; hence, they can be applied in low-tier hospitals and for point-of-care testing (POCT). The combination of anti-CCP antibody + AKA + GPI exhibited a specificity of 100% in the present study; hence, this combination can be used for a definitive diagnosis of RA.

Conclusions

In summary, the five serum markers were found to supplement each other in the diagnosis of RA diagnosis. Thus, combined testing of this panel of multiple markers increases the specificity of RA diagnosis substantially, thus facilitating the early diagnosis and treatment of RA.

Conflict of interest

None.

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