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ORIGINAL ARTICLE

Diagnostic performance of anti-RA33 antibody as a serological marker for rheumatoid arthritis

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Abstract

Introduction: Rheumatoid arthritis is diagnosed based on the 2010 Rheumatoid Arthritis Classification Criteria whereby rheumatoid factor and anti-citrullinated protein antibody are the serological markers included in these criteria. Anti-RA33 antibody has the potential to provide additional diagnostic value in rheumatoid arthritis. The aim of this study is to determine the diagnostic performance of anti-RA33 antibody as a serological marker for rheumatoid arthritis. *Material and methods:* Thirty-four patients with rheumatoid arthritis and 34 non-rheumatoid arthritis individuals were included in this cross-sectional study. Anti-RA33 antibody and rheumatoid factor were performed on all samples. *Results:* The sensitivity, specificity, positive and negative predictive value for anti-RA33 antibody and rheumatoid factor were 41.1%, 97.1%, 93.3%, 62.3% and 64.7%, 79.4%, 75.9%, 69.2% respectively. The overall sensitivity and specificity if either anti-RA33 antibody or rheumatoid factor are positive were 79.4% and 76.47% respectively. *Conclusion:* Anti-RA33 antibody showed good specificity and positive predictive value and could be considered as a potential serological marker for rheumatoid arthritis diagnosis.

Keywords: anti-RA33 antibody, rheumatoid arthritis, rheumatoid factor

INTRODUCTION

Rheumatoid arthritis is a chronic inflammatory disease that mainly affecting the synovial joint. The progression of the disease can lead to joint erosion and subsequently joint deformity. Although the main clinical manifestation involves the joint, the extra-articular involvement of the disease is well established. Rheumatoid arthritis is currently diagnosed according to the 2010 Rheumatoid Arthritis Classification Criteria.[1] The criteria are divided into four main subheadings that include joint involvement, duration of symptoms, serology and acute-phase reactants. Rheumatoid factor and anti-citrullinated protein antibody (ACPA) are included in this classification as the serological markers for the disease.

Rheumatoid factor is the first serology marker used to aid in diagnosing rheumatoid arthritis. Rheumatoid factors are autoantibodies directed against the fragment of IgG molecule and have different isotypes mainly of IgA, IgM and IgG. Laboratory testing detecting different isotypes provides different sensitivity and specificity for the diagnosis of rheumatoid arthritis.[2] It was suggested that measurement of these isotypes may increase 7- to 21-fold chance of making the diagnosis of rheumatoid arthritis serologically.[3] However, it is known that the specificity of rheumatoid factor is low. Rheumatoid factor can be found in other autoimmune diseases such as systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), systemic sclerosis and also in non-autoimmune conditions such as infections, liver cirrhosis and malignancy.[4] Thus, positive rheumatoid factor does not confirm rheumatoid arthritis diagnosis.

Anti-citrullinated protein antibody (ACPA) such as anti-cyclic citrullinated peptide on the other hand has been shown to provide

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comparable sensitivity but better specificity than rheumatoid factor. Avouac *et al*. showed the sensitivity for anti-CCP was 53% for anti-CCP1 and 68% for anti-CCP2 with specificity of the markers to be 95% and 96% for anti-CCP1 and anti-CCP2 respectively.[5] Another meta-analysis study by Nishimura *et al*. noted that anti-CCP sensitivity and specificity were 67% and 95% respectively.[6] A study in Malaysia noted that anti-CCP was the most prevalent autoantibody in rheumatoid arthritis.[7] Combination serology testing with both rheumatoid factors and anti-CCP was shown to improve the performance of serological markers for rheumatoid arthritis diagnosis. It was demonstrated that if all RFs isotypes and anti-CCP are done, IgM-RF and anti-CCP provide the highest sensitivity of 66.4% and 64.4% respectively while IgG-RF and anti-CCP provide the highest specificity of 91% and 97% respectively.[8]

Although both markers are good for the diagnosis of rheumatoid arthritis, yet they are rheumatoid arthritis patients with negative RF and ACPA. Thus, the introduction of anti-RA33 could possibly help to identify some of these patients. This may help to increase the sensitivity of serological tests for the diagnosis of the condition. Anti-RA33 was found among the rheumatoid arthritis patients whereby the autoantibody is a 33 kDA antigen that was first recognised in sera from rheumatoid arthritis patients using immunoblots from soluble nuclear extracts of HeLa cells.[9] Subsequently, many studies were conducted to determine the laboratory performance of this marker in rheumatoid arthritis. This study is conducted with the main objective to determine the sensitivity, specificity, positive and negative predictive value of anti-RA33 in the diagnosis of rheumatoid arthritis.

MATERIALS AND METHODS

Study population

Thirty-four rheumatoid arthritis patients were recruited from Rheumatology Clinic, Hospital Canselor Tuanku Muhriz, Universiti Kebangsaan Malaysia Medical Centre. All of them fulfilled the 2010 Rheumatoid Arthritis Classification Criteria and provided their consent to participate. Another 34 non-rheumatoid arthritis populations were recruited randomly from various clinics. These 34 non-rheumatoid arthritis patients consist of 15 patients with underlying SLE, 7 antenatal women, 3 with osteoarthritis and 9 healthy individuals. The samples were collected from 1st April 2016 until 31st July 2016.

Data collection

Basic demographic data that included age, gender, and ethnicity were collected from all participants. For rheumatoid arthritis population, the data about onset of the disease, duration of the disease, number, joint distribution and extra-articular manifestations were also gathered.

Laboratory analysis

Sample collection and preparation

Five milliliters of blood were collected from each patient. After the collection, the blood was placed into a gel and clot activator tube and transported to the Immunology Laboratory in the same hospital. In the laboratory, this tube was centrifuged at 3500 rpm for 10 minutes. Subsequently, the serum was transferred into another tube and stored at -20°C refrigerator until further used.

Antibody detection

Anti-RA33 antibody was detected by enzyme-linked immunosorbent assay (ELISA) method using the commercially available kit. For this research, anti-RA33 antibody was detected by using IMTEC-RA33 antibodies kit (Human, Wiesbaden, Germany). The anti-RA33 antibody detected by this kit was of IgG isotype. The ELISA was performed twice on each sample. The mean optical density (OD) was used to get the exact concentration of anti-RA33 antibody for each sample. We followed the ELISA steps as suggested by the manufacturer. The results were available as both quantitative and qualitative. The qualitative result was mainly used in this study purpose. The concentration of more than 25 IU/ml was taken as positive. This cut-off concentration was suggested by the manufacturer.

Rheumatoid factor was detected by latex agglutination method. The RF Direct Latex kit by Veda Lab (Alencon, Cedex, France) was used for the detection of rheumatoid factor in all samples. The result was available qualitatively. Samples that agglutinate within three minutes were considered as positive.

Acute phase reactants

C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were also measured in patients with rheumatoid arthritis. Both CRP and ESR results were available quantitatively and qualitatively. C-reactive protein is considered high if the concentration is more than 0.5 mg/dL. Erythrocyte sedimentation rate is considered high when the concentration is more than 20 mm/hour.

Statistical analysis

Data were analysed by using Statistical Packages for Social Sciences (SPSS) version 23. The relationship between anti-RA33 antibody with joint count, extra-articular manifestation and rheumatoid factor was analysed using the Chi-square test. The correlation between anti-RA33 antibody with CRP and ESR levels was analysed using Independent T-test. The p-value of < 0.05 was taken as significant for both analyses. The sensitivity, specificity, positive and negative predictive value of anti-RA33 antibody was calculated accordingly.

Research ethics approval

This study was approved by the Research and Ethics Committee of Hospital Canselor Tuanku Muhriz, Universiti Kebangsaan Malaysia Medical Centre with a research number of FF-2016-162.

RESULTS

Demographic and laboratory investigation results of rheumatoid arthritis patients

Thirty-four patients with rheumatoid arthritis and another 34 non-rheumatoid arthritis individuals were included in this study. The non-rheumatoid arthritis population consisted of 15 patients with underlying SLE, 7 pregnant women, 3 with osteoarthritis and 9 healthy individuals. The mean age for rheumatoid arthritis group was 57.62 years versus 52.4 years for non-rheumatoid arthritis population. Thirty of the rheumatoid arthritis patients were female. The majority of patients (76.5%) with rheumatoid arthritis complained of multiple joints pain that involves more than 10 joints pain. All except one patient had high ESR and 20 (58.8%) had high CRP levels. Table 1 showed the demographic and laboratory investigations of patients with rheumatoid arthritis.

TABLE 1: Demographic and laboratory results of patients with rheumatoid arthritis

Parameter	n (%)
Age	Mean 57.62 (+/- 10.33) years Range (26-81 years)
Gender:	
Male	4 (11.8)
Female	30 (88.2)
Ethnic:	
Malay	17 (50)
Chinese	11 (32.4)
India	5 (14.7)
Others	1 (2.9)
Joint count:	
More than 10 joints including one small joint	23 (67.6)
10 or less joints involved	11 (23.4)
Extra-articular manifestations:	
Yes	16 (47.1)
No	18 (52.9)
Duration of disease:	
Early (<2 years)	4 (11.8)
Establish	30 (88.2)
C-reactive protein:	
High (>0.05 mg/dL)	20 (58.8)
Normal	14 (41.2)
Erythrocyte sedimentation rate (ESR):	
High (>20 mm/hour)	33 (97.1)
Normal	1 (2.9)
Rheumatoid factor:	
Positive	22 (64.7)
Negative	12 (35.3)
Anti-RA33 antibody:	
Positive	14 (41.2)
Negative	20 (58.8)

Sensitivity, specificity, positive and negative predictive value of anti-RA33 antibody and rheumatoid factor

Anti-RA33 antibody was positive in 15 patients, 14 in rheumatoid arthritis and 1 in non-rheumatoid arthritis populations. This one non-disease population sample had underlying osteoarthritis. Anti-RA33 antibody showed the sensitivity, specificity, positive and negative predictive value of 41.1%, 97.1%, 93.3% and 62.3% respectively.

Rheumatoid factor was positive in 29 samples, 22 from rheumatoid arthritis and 7 from non-rheumatoid arthritis groups. The seven non-rheumatoid arthritis samples that were positive for rheumatoid factor include five patients with SLE and two healthy individuals. Thus, the calculated sensitivity, specificity, positive and negative predictive value for rheumatoid factor was 64.7%, 79.4%, 75.9% and 69.2% respectively.

It was noted that 9 rheumatoid arthritis patients were positive for both rheumatoid factor and anti-RA33 antibody, 13 positives for rheumatoid factor only, 5 positives for anti-RA33 antibody only and 7 were negative for both markers. The sensitivity, specificity, positive and negative predictive value if either one of the markers was positive were 79.4%, 76.47%, 77.14% and 78.79% respectively. These results were shown in Table 2.

The association between anti-RA33 antibody with joint count, extra-articular manifestation, duration of the disease and rheumatoid factor

Table 3 showed the association between anti-RA33 antibody with parameters such as joint count, extra-articular manifestation, duration of the disease and rheumatoid factor. There was no significant association between anti-RA33 antibody with those parameters ($p>0.05$).

The association between anti-RA33 antibody with the concentration of ESR and CRP

Table 4 showed the association between anti-RA33 antibody with the concentration of ESR and CRP. Again, there was no significant relationship between anti-RA33 antibody with the ESR and CRP levels ($p>0.05$).

DISCUSSION

This study showed that anti-RA33 antibody performed better than rheumatoid factor with superior specificity (97.1% versus 79.4%) and positive predictive value (93.3% versus 75.9%). We demonstrated that the combination of these two serology markers improved the overall sensitivity than doing either marker alone (76.4% versus 41.1% for anti-RA33 antibody and 64.7%

TABLE 2: Sensitivity, specificity, positive and negative predictive value

Parameter	Rheumatoid Arthritis	Non-Rheumatoid Arthritis	Analysis
Rheumatoid factor:			
			Sensitivity: 64.7%
Positive	22	7	Specificity: 79.4%
Negative	12	27	PPV: 75.9%
			NPV: 69.2%
Anti-RA33 antibody:			
			Sensitivity: 41.1%
Positive	14	1	Specificity: 97.1%
Negative	20	33	PPV: 93.3%
			NPV: 62.3%
Combination Rheumatoid factor/Anti-RA33 antibody:			
			Sensitivity: 79.4%
			Specificity: 76.47%
Positive	27	8	PPV: 77.14%
Negative	7	26	NPV: 78.79%

PPV: Positive predictive value, NPV: Negative predictive value

TABLE 3: The association between anti-RA33 antibody with joints count, extra-articular manifestation of rheumatoid arthritis, duration of the disease and rheumatoid factor

Parameter	Anti-RA33 Antibody		P-value*
	Positive	Negative	
Joints count:			
More than 10 joints including 1 small joint	9	14	1.0
10 or less joints involved	5	6	
Extra-articular manifestations:			
Yes	8	8	0.487
No	6	12	
Duration of disease:			
Early (< 2 years)	1	3	0.627
Established (³ 2 years)	13	17	
Rheumatoid factor:			
Positive	9	13	1.0
Negative	5	7	

*Analysed by Fisher exact chi-square test, p-value <0.05 is significant.

for rheumatoid factor) albeit lower specificity to performing anti-RA33 antibody alone (79.4% versus 97.1%).

The main advantage of anti-RA33 antibody lies in its superior specificity than rheumatoid factor. Anti-RA33 antibody was detected in only one out of 170 non-rheumatoid arthritis population in an earlier study.[9] Subsequently, more studies that had been carried out showed the specificity of this marker was noted to be more than 80%.[10-14] Our result of anti-RA33 specificity of 97.1% was in agreement with the previous works. However, we also noted one study that described a very low specificity at 20%.[15]

We demonstrated that the sensitivity of anti-RA33 antibody was lower than rheumatoid factor. In this study we noted that the sensitivity of anti-RA33 antibody was 41.1%. A study conducted among patients with rheumatoid arthritis in Saudi noted that anti-RA33 antibody was present in only 7.3%.[10] Other studies showed the sensitivity

TABLE 4: The correlation of anti-RA33 antibody with ESR and CRP values

	Anti-RA33 Antibody	N	Mean +/- SD	P-value*
ESR	Positive	14	59.29+/- 32.08	
	Negative	10	55.9+/-26.81	0.740
CRP	Positive	14	1.84+/-2.69	
	Negative	10	1.03+/-1.77	0.297

*Independent T test was used to analyse this data. P-value of <0.05 is significant.

of anti-RA33 antibody was between 14.5% to 36%.[9,11-14,16] We showed a higher sensitivity of anti-RA33 antibody among our patients with rheumatoid arthritis in comparison to previous studies.

It was noted that anti-RA33 antibody could also be found in other systemic autoimmune diseases. Anti-RA33 antibody was positive in 10.3% of patients with spondyloarthropathy, 16.9% of patients with systemic sclerosis and 8% of patients with SLE.[17] We reported one individual with underlying osteoarthritis positive for anti-RA33 antibody but none in those with underlying SLE.

This study also showed when both anti-RA33 antibody and rheumatoid factor were performed together, the sensitivity was improved to 79.4% if either one of the markers was positive. This performance was better than anti-RA33 antibody and rheumatoid factor sensitivity alone. It was described previously that stepwise model testing that included all serological tests for rheumatoid arthritis namely rheumatoid factor, ACPA and anti-RA33 antibody helped to increase overall sensitivity.[11]

We failed to exhibit any significant association between positive anti-RA33 antibody with the number of joint involved, duration of the disease, rheumatoid factor, ESR and CRP levels. Earlier studies showed anti-RA33 antibody correlates with rheumatoid factor.[11,12,17] There were several other studies that demonstrated a correlation between anti-RA33 antibody with disease duration and acute phase reactants. Al-Mughales found that changes in anti-RA33 antibody concentration did correlate with CRP levels.[10] Other study showed significant negative correlation between anti-RA33 antibody with disease duration indicating that this antibody has perhaps occurred early in rheumatoid arthritis although no difference was found in terms of concentration of the antibody in early and established rheumatoid arthritis.[17] Anti-RA33 antibody was also suggested to help in discriminating those with early rheumatoid arthritis from other forms of arthritis.[18] We did not show any significant correlation possibly due to the fact that the majority of these patients were established rather than early stage of rheumatoid arthritis.

We noticed some limitations in our study as we did not take into account the disease activity of the patients at the time of recruitment that may be useful for correlation study. We also only managed to take the sample at one single point rather than at multiple points to better analyse the correlation between anti-RA33 antibody levels and disease activity. Further study is needed to evaluate the prognosis of those rheumatoid arthritis patients with positive anti-RA33. We also did not include anti-CCP in this study that probably may increase overall sensitivity of the serological marker in rheumatoid arthritis diagnosis if all markers were done together. We showed good performance of anti-CCP in previous work with high specificity and good sensitivity when compared to rheumatoid factor.[19]

In conclusion, this study showed that anti-RA33 antibody provides additional diagnostic value for serological diagnosis of rheumatoid arthritis with better specificity and positive predictive value. Combination or stepwise testing of rheumatoid factor followed by anti-RA33 antibody enhances overall sensitivity of serological markers for rheumatoid arthritis.

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