ORIGINAL ARTICLE

Anti-RA 33: A Marker of Good Prognosis in Seronegative Rheumatoid Arthritis

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ABSTRACT

Objective: *Autoantibodies are evident in the early stages of rheumatoid arthritis (RA) and play important roles in diagnosis. The aim of this study was to investigate the diagnostic capability and extent of anti-RA 33 positivity and clinical characteristics in patients with RA.*

Methods: *We included 67 RA patients and 20 healthy subjects in our study. Duration of symptoms, duration of disease, the extent of delay in diagnosis, episodes of clinical remission, and type and number of disease-modifying antirheumatic drugs (DMARDs) taken were noted. To evaluate quality of life, the Health Assessment Questionnaire (HAQ) Disability Index (consisting of 20 questions) was applied. Disease activity was evaluated with Disease Activity Score (DAS) 28. The laboratory assessments included erythrocyte sedimentation rate, C-reactive protein level and serologic assessments for rheumatoid factor, anti-cyclic citrullinated protein and anti-RA 33.*

Results: *The mean disease duration was 14.56 months. A total of 38 (56.7%) patients were positive for anti-RA 33 antibodies. Twenty-four (63%) of patients positive for anti-RA 33 were clinically in remission. A negative correlation was evident between anti-RA 33 positivity and number of DMARDs taken and HAQ score (*$r = -0{,}766$, $p < 0.001$; $r = -0{,}737$, $p < 0.001$*). A positive correlation was evident between anti-RA 33 positivity and DAS 28 score (*$r = 0.287$, $p = 0.019$*).*

Conclusion: *Anti-RA 33 antibodies have poor diagnostic capability in patients with RA. Anti-RA 33 antibodies may exert helpful effects determining prognosis in established RA patients.*

Keywords: Inflammation, prognostic factor, rheumatoid arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease characterized by the presence of autoantibodies and autoreactive T cells in peripheral blood and synovial fluid. Autoantibodies are evident in the early stages of disease and play important roles in diagnosis. Autoantibodies continue to be evident for some years. Rheumatoid factor (RF), the oldest described determinant of RA, is an autoantibody targeting the Fc portion of IgG. Serological determinants of RA assumed greater importance when the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria were revised in 2010. Positivity for anti-citrullinated protein antibodies (ACPA) was considered to be significant in this criterion (1). Anti-citrullinated protein antibodies are present in the sera of 80%–90% of RA patients.

In some studies, ACPA-positivity was more specific for RA diagnosis than was RF-positivity, with specificity approached 90% (2, 3). Several studies have shown that

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anti-RA 33 antibodies are present in early stages of RA, and the levels thereof did not correlate with those of RF or ACPA (4). Anti-RA33 antibodies are also produced in the tumour necrosis factor (TNF) transgenic mouse model of RA, suggesting that proinflammatory cytokines can independently trigger a breakdown of tolerance to this protein (5). An algorithm involving anti-RA 33, RF and anti-cyclic citrullinated protein (anti-CCP) antibody levels can be used to predict which patients with early-stage synovitis will progress to erosive RA, although the algorithm is not especially sensitive to or specific for RA when used in isolation (6). In the present study, we investigated the diagnostic capacity and the extent of anti-RA 33 positivity in RA patients, and the clinical characteristics of such patients in the context of ACR/EULAR 2010 criteria.

SUBJECTS AND METHODS

Patients were selected from the registry of our rheumatology outpatient clinics, which consisted of almost 400 RA patients. Based on the patients' charts, we contacted subjects with the following inclusion criteria: (a) those who did not have cancer or any haematological abnormality, (b) those who were not pregnant or were in the recent post-partum period (6 months) and (c) those who accepted the term of the study.

We enrolled 67 RA patients who fulfilled the ACR/EULAR 2010 RA classification criteria and who were followed up in the rheumatology outpatient clinic of the Medicine Faculty Hospital. Sex-age matched 20 healthy subjects were recruited from the relatives of health professionals. The ACR/EULAR RA classification criteria feature evaluation of (a) joint involvement, (b) RF and anti-CCP levels, (c) acute-phase reactant levels and (d) duration of symptoms (1). A total of 40 patients were seropositive (RF-anti-CCP positive or RF positive, anti-CCP negative or RF negative, anti-CCP positive), and 27 patients seronegative for RF and anti-CCP antibodies. Informed consent was obtained from all patients. The study protocol was approved by the Ethics Committee of Sakarya University.

Age, gender, body mass index and smoking habits were recorded. Duration of symptoms, duration of disease, the extent of delay in diagnosis, epiosodes of clinical remission, number of disease-modifying anti-rheumatic drugs (DMARDs) taken, and relevant family history were noted. Disease Activity Score (DAS) 28 remission criteria, involving C reactive protein (CRP), swollen and tender joint counts, and patient's global health assessment, were used to determine whether the disease is in remission. A score of DAS 28 between 2.6 and 3.2 indicates low disease activity, 3.2 and 5.1 moderate and > 5.1 high disease activity (7).

To evaluate quality of life, the Health Assessment Questionnaire (HAQ) disability index (consisting of 20 questions) was applied (8).

Venous blood samples were collected after an overnight fast for the laboratory tests. Serum RA 33 was determined using respective (monoclonal/polyclonal) antibodies (both from Eastbiopharm, Hangzhou, China) using the Triturus automated enzyme-linked immunosorbent assay (ELISA) equipment (Grifols, Lillyvale Ave, LA, USA). Erythrocyte sedimentation rate (ESR) was determined immediately after blood collection using a Greiner SRS 20/II instrument (Vacuette Greiner, Kremsmunster, Austria). C-reactive protein (inflammatory markers) levels were determined by nephelometric methods using an IMAGE 800 analyzer (Beckman Coulter Inc., Brea, CA, USA).

Rheumatoid factor was measured by nephelometry; a level of 20 U/ml was considered positive (as suggested by Beckman Coulter IMMAGE® Immunochemistry Systems). Anti-CCP antibodies were measured via ELISA, and a result was considered positive if the level was above a cut-off of five arbitrary units (as suggested by Abbott ARCHITECT i1000SR). Anti-RA33 antibodies were assessed via ELISA and a result was considered positive if the level was over 25 IU/ml (as suggested by the HUMAN Imtec Product Line).

Statistical analysis

SPSS statistical software (IBM SPSS version 20.0, IBM Corp., Armonk, NY, USA) was used for all statistical analyses. Quantitative variables (clinical or laboratory) are given as means ± SDs or as ranges. Correlations between clinical and laboratory parameters, and autoantibody levels, were analysed using Pearson's correlation test. Receiver operating characteristic (ROC) analysis was then performed in order to assess the anti-RA 33 antibodies and to obtain estimates of sensitivities, specificities, positive predictive values and negative predictive values using the clinical diagnosis of RA (ACR/EULAR 2010 diagnostic criteria) as the reference standard. A p value less than 0.05 was considered statistically significant.

RESULTS

We included 40 seropositive RA patients, 27 RF-negative anti-CCP-negative and 20 healthy subjects in our study. The clinical and laboratory characteristics of all patients

and healthy subjects are shown in Table 1. Intragroup anti-RA 33 distribution was homogeneous in terms of demographic characteristics ($p > 0.05$). Of all patients, 20.9% were clinically in remission. A total of 38 (56.7%) patients were positive for anti-RA 33 antibodies. One (5%) healthy subject was positive for anti-RA 33 antibodies. We found statistically significant difference between patients and healthy subjects for anti-RA 33 positivity ($p = 0.031$) (Table 1).

We found no significant difference in duration of symptoms, the duration of diagnosis or delay in diagnosis between patients with and without anti-RA 33 antibodies ($p = 0.843$, $p = 0.740$ and $p = 0.605$, respectively). Other rheumatological features in RA patients with or without anti-RA 33 are shown in Table 2.

The ratio of patients with clinical remission was 20.9% (n = 14), and low disease activity, moderate disease activity and high disease activity were 29.8% (n = 20), 19.5% (n = 13) and 29.8 (n = 20), respectively. Ten (26%) patients positive for anti-RA 33 were in remission, as were 13% (n = 4) of those negative for anti-RA 33. A significant (but weak) correlation was observed between positivity for anti-RA 33 and clinical remission ($p = 0.04$, $r = 0.265$). Anti-RA 33 positivity was more frequent in seronegative RA patientscompared to seropositive RA patients, but this difference was not statistically significant ($p = 0.09$, 62% *vs* 52%).

Table 1: Clinical and laboratory characteristics of 67 study patients with RA and 20 healthy subjects

	RA patients (n = 67)	Healthy subjects (n = 20)	*p* value
Age, mean ± SD, [IQR], years	50.25 ± 14.5 [52.5]	49.32 ± 12.2 [49.12]	0.124
Sex, % women	80.6	80	0.238
BMI, mean ± SD, [IQR], kg/cm²	28.82 ± 2.41 [28.7]	27.64 ± 2.28 [27.5]	0.387
Cigarette smoking, % patients	32.8	30	0.109
Duration of symptoms, mean ± SD, [IQR], months	18.53 ± 11.43 [19.57]	NA	NA
Disease duration, mean ± SD, [IQR], months	14.56 ± 12.12 [15.50]	NA	NA
Delay in diagnosis, mean ± SD, [IQR], months	5.53 ± 3.89 [4.50]	NA	NA
DAS 28, mean ± SD, [IQR]	3.86 ± 1.13 [3.95]	NA	NA
Tender joints, mean ± SD, [IQR]	3.89 ± 2,75 [4.02]	NA	NA
Swollen joints, mean ± SD, [IQR]	2.97 ± 2.13 [2.85]	NA	NA
HAQ total scores, mean ± SD, [IQR]	3.56 ± 2.54 [3.97]	NA	NA
Anti-RA 33 positivity, % patients	56	5	0.005
Anti-RA33, mean ± SD, [IQR], IU/ml	37.99 ± 38.68 [30.01]	18.25 ± 9.29 [16.5]	0.031
ESR, mean ± SD, [IQR], mm/h	41.65 ± 18.41 [40.50]	15.35 ± 4.35 [14.5]	0.001
CRP, mean ± SD, [IQR], mg/L	18.96 ± 12.21 [19.50]	2.55 ± 1.15 [2.50]	0.002

IQR= interquartile range; RA = rheumatoid arthritis; anti-RA 33 = heterogeneous nuclear ribonucleoprotein A2; BMI = body mass ındex; CRP = C-reactive protein; DAS 28 = Disease Activity Score 28; ESR = erythrocyte sedimantation rate; HAQ = Health Assessment Questionnaire; NA = not assessed.

Table 2: Comparison of rheumatological features in RA patients with or without anti-RA 33

	Anti-RA 33 positive group (n = 38)	Anti-RA 33 negative group (n = 29)	*p* value
Age (mean ± SD), years	48.74 ± 13.27	53.38 ± 14.89	0.111
Sex, % women	81.6	86.2	0.615
BMI, kg/cm²	28.83 ± 2.39	29.0 ± 2.30	0.690
Cigarette smoking, % patients	44.7	34.5	0.400
Duration of symptoms (mean ± SD), months	42.32 ± 29.28	41.34 ± 20.91	0.652
Disease duration (mean ± SD), months	36.42 ± 25.77	36.28 ± 20.60	0.868
Delay in diagnosis (mean ± SD), months	5.89 ± 4.65	5.07 ± 2.81	0.617

BMI = body mass index.

Methotrexate has been the first choice in both groups. In anti-RA 33-positive group, four patients with TNF blockers were included. Otherwise, there was no difference between the types of other DMARDs in each group. The names of DMARDs in anti-RA 33-positive group were as follows: methotrexate, leflunomid, hydroxychloroquine sulphate and sulphasalazine and the ratio was 62%, 42%, 31% and 24%, respectively. The names of DMARDs in anti-RA 33-negative group were as follows: methotrexate, leflunomid, hydroxychloroquine sulphate, sulphasalazine, and TNF blockers (two adalimumab, two etanercept) and the ratio was 73%, 59%, 42%, 42%, and 13%, respectively.

If the cut-off of anti-RA33 was set at 25.0, patients with RA had 55% specificity and 20% sensitivity. The positive and negative predictive values of the test were 50% and 42%, respectively. When only established RA patients (disease duration >12 months) were evaluated, the specificity, sensitivity, positive and negative predictive values of the test were 81%, 50%, 60% and 40%, respectively.

Figure 1 shows the comparative ROC curve of the two mentioned models. Area under the ROC curve was 0.836 (95% CI: 0.75, 0.92) for all RA patients and 0.965 (95% CI: 0.92, 1.00) for established RA patients. Best cut-off point for both models was estimated to be > 25.

The average HAQ of all patients was 3.20. The HAQ scores of patients positive and negative for anti-RA 33 were respectively 1.74 ± 1.28 and 4.97 ± 1.72 (means and standard deviations). A significant, strong negative correlation was evident between anti-RA 33 titres and HAQ score ($p = 0.000$, $r = -0.737$). There was a negative

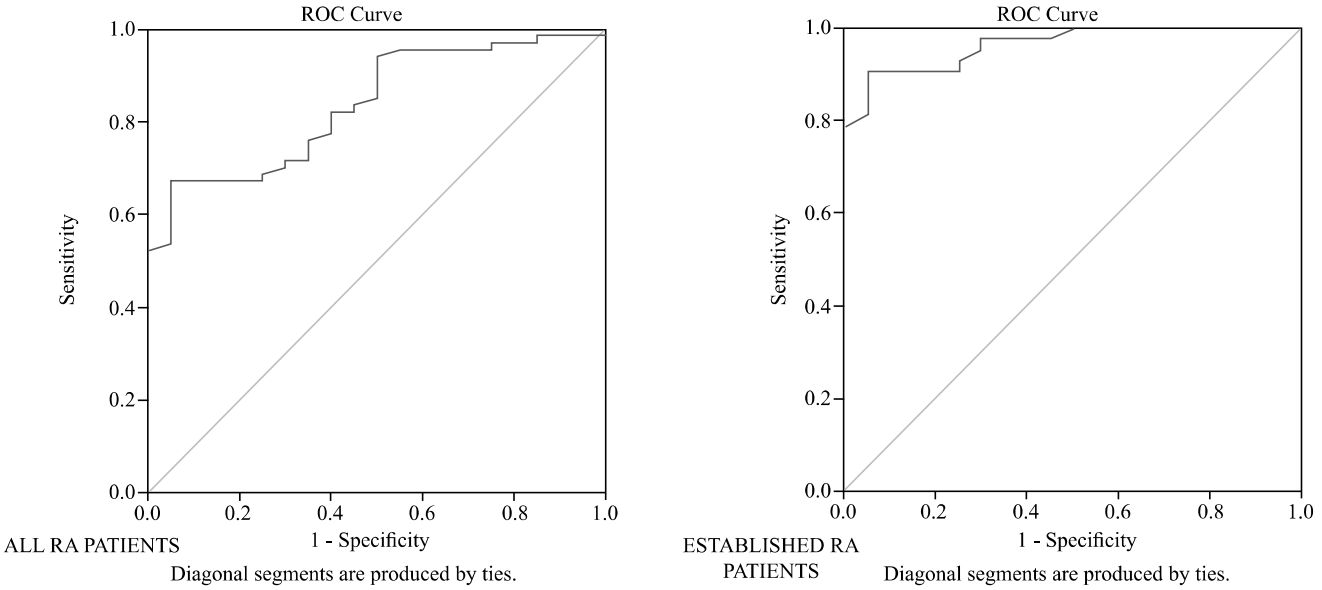

Fig. 1: Sensitivity and specificity of anti-RA 33 in established and all RA patients. RA = rheumatoid arthritis; ROC = receiver operating characteristic.

correlation between anti-RA 33 titres and number of DMARDs taken ($p = 0.006$, $r = -0.333$) (Fig. 2).

The average DAS 28 of all patients was 2.95. The DAS 28 scores of patients positive and negative for anti-RA 33 were, respectively, 3.55 ± 1.15 and 2.77 ± 1.23 (means and standard deviations). A significant weak positive correlation was evident between anti-RA 33 titres and DAS 28 score ($p = 0.019$, $r = 0.287$) (Fig. 3).

A subanalysis of patients with anti-RA 33-positive or anti-RA 33-negative revealed a significant difference in HAQ scores and number of DMARDs taken ($p < 0.05$) (Table 3).

DISCUSSION

Many antibodies have been used as markers for RA diagnosis and to reflect RA pathogenesis (9). To the best of our knowledge, the most specific antibody in these contexts is anti-CCP.

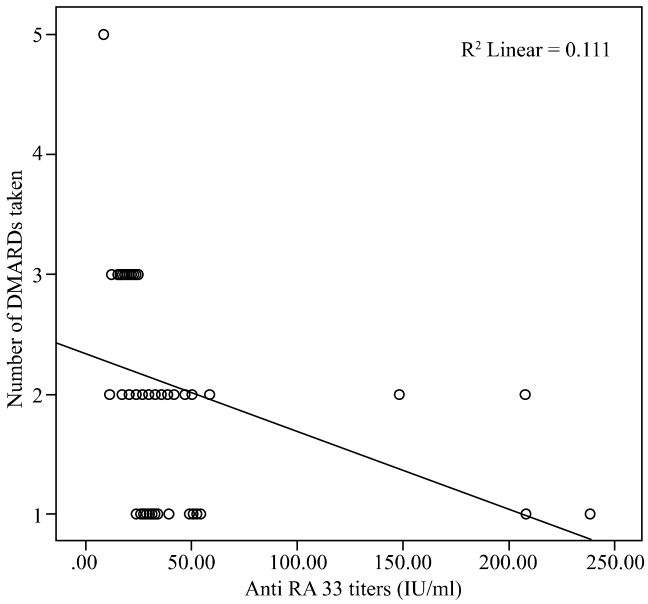

Fig. 2: Relationship of anti-RA 33 titres and number of DMARDs taken in study patients. DMARD =disease-modifying antirheumatic drug; RA = rheumatoid arthritis.

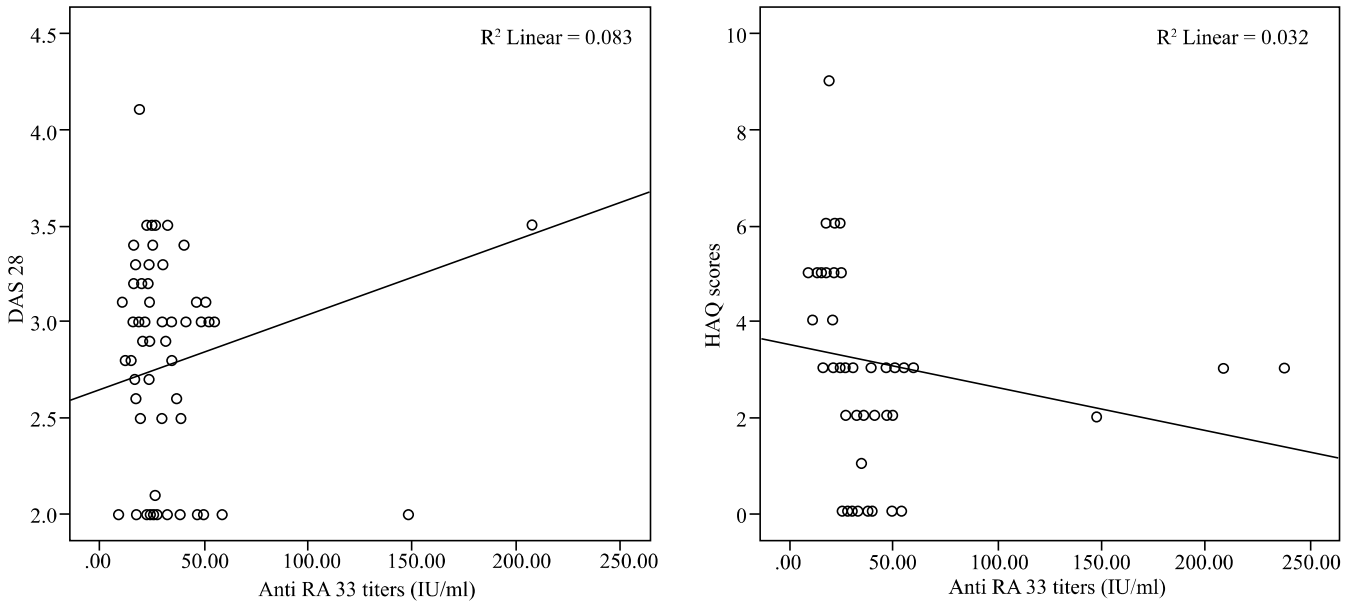

Fig. 3: Relationship of anti-RA 33 titres and HAQ and DAS 28 scores. DAS = Disease Activity Score; HAQ = Health Assessment Questionnaire; RA = rheumatoid arthritis

Table 3: Demographic and clinical data in patients with RA with or without anti-RA 33

	Anti-RA 33 positive group (n = 38)	**Anti-RA 33 negative group (n = 29)**	***p* value**
HAQ scores, mean ± SD	1.74 ± 1.28	4.97 ± 1.72	< 0.001
Number of DMARDs taken, mean ± SD	1.58 ± 0.54	2.80 ± 0.61	< 0.001
Presence of clinical remission, %	63.2	44.5	0.215

RA = rheumatoid arthritis; HAQ = Health Assessment Questionnaire; DMARD = disease-modifying antirheumatic drug.

Further research showed more specific markers such as anti-mutated citrullinated vimentin and anti-CCP without RF in diagnosis of RA (10–12). Patients diagnosed with arthritis in clinical practice, but who are not RF- or anti-CCP-positive may be difficult to diagnose. Of antigens expressed by RA patients, heterogeneous nuclear ribonucleoprotein A2 (hnRNP A2) (RA 33) levels seem to be correlated with both RA development and pathogenesis. RA 33 is not a citrullinated peptide and, thus, differs from ACPA-group antigens. The antigen first named RA 33 is a protein of 36 kDa associated with mRNA metabolism, possibly active in pre-mRNA addition, mRNA transport and regulation of translation (4).

The activities of anti-RA 33 in RA patients are poorly understood. A few studies have indicated that anti-RA 33 occurs at comparable frequencies in patients suffering from systematic lupus erythematosus or mixed connective tissue disease, and about 33% of RA patients are antigen-positive. However, compared to mixed connective tissue diseaseand systemic lupus erythematosus patients, RA patients mount a more restricted immune response to the spliceosome (13). Such patients have antibodies to hnRNP proteins, particularly hnRNP-A2 (RA33), but not to small nuclear RNPs (14). Anti-RA 33 antibody levels remain stable in the course of RA (15, 16). Anti-RA 33 antibodies react with a nuclear antigen. In recent years, it has been suggested that anti-Sa autoantibody levels (Sa is a member of the ACPA family) should be measured in patients negative for anti-CCP (17). However, extensive family evaluations may not yield new data and will thus incur unnecessary costs. It is more logical to measure the levels of autoantibodies against nuclear structures, except citrullinated proteins, to evaluate cases negative for anti-CCP and who do not express known relevant proteins. Autoantibodies in patients negative for RF were studied in early RA research performed in 2000; 25.4% expressed anti-ceratin antibodies reacting with the citrullinated protein filaggrin. In patients negative for RF, 23.9% were positive for anti-RA 33 autoantibodies (18).

In recent years, we found two studies in the literature about anti-RA 33 and RA (19, 20). The aim of these studies was to calculate the sensitivity and specificity of anti-RA 33 in patients with RA. Lashkari *et al* (19) indicated that anti-RA33 test had 98% sensitivity, 20% specificity, 50% positive predictive value and 90% negative predictive value. They pointed out that anti-RA 33 could be valuable, when serologic tests were negative.

Other prediction of this study was the prognostic value of anti-RA 33 in patients with RA.

However, Al-Mughales *et al* (20) showed that anti-RA33 antibodies had substantially low sensitivity (7.32%) and high specificity (95.12%) compared with RF. The most important of results of this study was that anti-RA33 autoantibodies seem to be not representing as an important additional immunodiagnostic marker in patients with established RA. However, this autoantibody could have been a candidate for established RA and less aggressive RA.

In the present study, we found that the anti-RA 33 positivity level was 56.7% in such patients. We found similar results with Al-Mughales *et al* study. At the same time, we showed the increase in the specificity of anti-RA 33 in the late stage of RA. According to our results, anti-RA 33 cannot be evaluated to diagnose in early stage of RA because of low sensitivity and specificity. When the diagnosis in doubt at late stage, anti-RA 33 might come to mind.

Steiner *et al* (21) showed that anti-hnRNP-A/B autoantibodies served not only as valuable diagnostic markers but the levels thereof may also afford additional insights into the pathogenic mechanisms of autoimmune rheumatic diseases. Also, in recent years, T-cell reactivity to HnRNP-A2 has been observed in nearly 50% of RA patients (22). As RA 33 is a nuclear antigen, patients expressing RF and anti-CCP may not express antibodies. It is thus logical to measure anti-RA 33 antibody levels to aid diagnosis in established RA patients or who do not express RF or anti-CCP. The positivity rate was 56.7% in such patients of our study, supporting our above contention. Anti-RA33 data, despite the limited sensitivity thereof, may be useful in established RA patients.

Anti-CCP antibodies are associated with poor prognoses in terms of radiographic joint damage and functional outcomes in patients seronegative for RA, and also those with recent-onset RA (23). We have shown that, generally, patients positive for anti-RA 33 have good prognoses. The prognostic importance of anti-RA 33 status has been evaluated in only a single study performed in 2005. This study showed that anti-RA33 assessment allowed identification of patients with good prognoses who responded well to treatment with DMARDs (5). Contrary to current study, Meyer *et al* (24) claimed that anti-RA 33 positivity tends to occur in RA patients with erosive RA and RA patients who have high ESR level (47.6% *vs* 24.4% and 42.8% *vs* 29.4%). There was no data on anti-CCP positivity in this study. According to ACR/EULAR 2010 RA classification criteria, titre and positivity of anti-CCP are so valuable. As well there are numerous studies on anti-CCP positivity that serves as a poor prognostic marker in patients with RA (25, 26).

The main strength and originality of our study are the relationship between clinical parameters (disease activity, quality of life, joint examination) and anti RA 33 status in patients with RA in comparison to healthy subjects. In the present study, we found a strong negative correlation between anti-RA 33 positivity and HAQ scores, showing that the quality of life is better in such patients. Also, we found lower disease activity in anti-RA 33 positive group. The amount of DMARDs required to suppress disease activity was less than that required by anti-RA 33-negative patients. Furthermore, the extent of clinical remission seemed to be greater in RA patients in the late period of anti-RA 33-positivity. Together, the data allow us to conclude that the prognosis of RA patients with anti-RA 33 antibodies is better than that of others.

There are some limitations. One of the limitation of our work is that the number and type of patient groups obtained in the current study are not sufficient to explain this results. The study population has limited number.

CONCLUSİON

Anti-RA 33 antibodies are associated with good outcome in RA patients though poor diagnostic capability. Further studies should evaluate anti-RA 33 status in early and established RA patients in larger groups, and adjust the sensitivity and specificity of the test.

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