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

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Running head: Anti-RA 33 and Aheumatoid Arthritis

Synopsis: We investigated the diagnosis capability and extent of Anti-RA 33 positivity and clinical characteristics in patients with RA. We highligths a strong negative corrrelation between Anti-RA 33 positivity and the quality of life. Anti-RA 33 antibodies may exert helpful effects determining prognosis in established RA patients though have poor diagnostic capability.

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 diagnosis 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, epiosodes of clinical remission, 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 (ESR), C-reactive protein (CRP) level, and serologic assessments for RF, anti-CCP 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, 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

INTRODUCTİON

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 Antibodies (ACPA) was considered to be significant in this criteria (1). ACPAs 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; the specificity approached 90% (2,3). Several studies have shown that 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 Tumor 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–citrullinated protein antibody (anti-CCP) 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 diagnosis 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.

MATERİAL AND METHOD

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: (i) those who did not have cancer, any hematological abnormality (ii) those who were not pregnant or were in the recent post-partum period (6 months), iii) 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) rheumatoid factor (RF) and anti-citrullinated protein antibody (anti-CCP) levels; c) acute phase reactant levels; and, d) duration of symptoms (1). 40 patients were seropositive (RF-Anti CCP positive or RF positive, Anti CCP negative or RF negative, anti CCP positive), 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 antirheumatic 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 in remission. A score of DAS28 between 2.6-3.2 indicates low disease activity, 3.2-5.1 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 were determined using respective (monoclonal/polyclonal) antibodies (both from Eastbiopharm, Hangzhou, China) using the Triturus automated enzyme-linked immunosorbent assay (ELISA) equipment (Grifols, Lillyvale Ave, Los Angeles, USA). Erithrocyte sedimentation rate (ESR) was determined immediately after blood collection using a Greiner SRS 20/II instrument (Vacuette Greiner, Kremsmunster, Austria). CRP (inflammatory markers) levels were determined by nephelometric methods using an IMAGE 800 analyzer (Beckman Coulter Inc., Brea, CA, USA).

RF 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 Enzyme Linked Immunosorbent Assay (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) was used for all statistical analysis. Quantitative variables (clinical or laboratory) are given as means±SDs, or as ranges. Correlations between clinical and laboratory parameters, and autoantibody levels, were analyzed using Pearson's correlation test. 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 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. Intra-group 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. A total of 1 (5%) healthy subjects were 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 was shown in table 2.

The ratio of patients with clinical remission were 20.9% (n= 14), low disease activity, moderate disease activity and high disease activity were 29.8% (n= 20), 19.5% (n= 13) 29.8 (n=20), respectively. Ten (26%) of 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 were more frequent in seronegative RA patients compared to seropositive RA patients but this difference was not statistically significance (p= 0.09, 62% versus 52).

Methotrexate has been the first choice in both groups. In anti-RA 33-positive group included 4 patients using TNF blockers. 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 the following; methotrexate, leflunomid, hydroxychloroquine sulphate, sulphasalazine and the ratio was 62%, 42%, 31%, 24%, respectively. The names of DMARDs in anti-RA 33-negative group were as the following; methotrexate, leflunomid, hydroxychloroquine sulphate, sulphasalazine and TNF blockers (2 adalimumab, 2 etanercept) and the ratio was, 73%, 59%, 42%, 42% and 13%, respectively.

If the cutoff of Anti-RA33 was set at 25.0, it had 55% specificity and %20 sensitivity for recognizing patients with RA. The positive and negative predictive values of the test were 50% and 42%, respectively. When we evalauted only established RA patients (disease duration>12 month), the specificity, sensitivity, positive and negative predictive values of the test were %81, 50%, 60%, 40, respectively.

Figure 1 shows the comparative ROC curve of the 2 mentioned models. Area under the ROC curve was 0.836 (95% CI: 0.75–0.92) for all RA patients and 0.965 (CI 95%: 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 titers and HAQ score (p=0.000, r= -0.737).

The average DAS28 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 titers and DAS 28 score (p=0.019, r= 0.287) (Figure 3).

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

DİSCUSSİON

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

Further research showed more spesific markers such as anti-MCV and anti CCP without RF in diagnosis of RA (10,11,12). 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, heterogenous 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 MCTD and SLE 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 antibodies levels remains 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 2 studies in the literature about anti RA 33 and RA (19, 20). The aim of these studies was to calculate the sensitivity, specifity 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.

But Al-Mughales et al (20) showed that Anti-RA33 antibodies had substantially low sensitivity (7.32%) and high spesifity (95.12%)compared with RF. The most important of results of this study was that anti-RA33 autoantibodies seems to be not representing as an important additional immunodiagnostic marker in patients with established RA. Hovewer, this autoantibody could have been to be a candidate for established RA and less aggresive RA.
In the present study, we found that the Anti-RA 33 positivity level was 56.7% in such patients. We found similiar results with Al-Mughales et al study. At the same time, we showed the increasing of the spesificity of anti RA 33 in late stage of RA. According to our

results, anti RA 33 can not be evaluated to diagnose in early stage of RA because of low sensitivity and spesificity. When the diagnosis in doubt at late stage, anti RA33 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 tended to be occuring 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, titer and positivity of anti-CCP is so valuable. As well there are numerous studies on anti-CCP positivity 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 comparison to healthy subjects. In present study, we found a strong negative corrrelation between Anti-RA 33 positivity and HAQ scores, showing that the quality of life is better in such patients. Also, we found lower disease acitivity 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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Table 1. Clinical and laboratory characteristics of 67 study patients with rheumatoid arthritis and 20 healthy subjects

	RA patients (n=67)	Healthy subjects (n=20)	P value
Age, mean [IQR] ± SD, years	50.25 ± 14.5 [52.5]	49.32 ± 12.2 [49.12]	0.124
Sex, % women	80.6	80	0.238
BMI, mean [IQR] ± SD, 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 [IQR] ± SD, months	18.53 ± 11.43 [19.57]	NA	NA
Disease duration, mean ± [IQR] SD, months	14.56 ± 12.12 [15.50]	NA	NA
Delay in diagnosis, mean [IQR] ± SD, months	5.53 ±3.89 [4,50]	NA	NA
DAS 28, mean [IQR]± SD	3,86 ± 1.13	NA	NA
Tender joints, mean [IQR]± SD	3,89 ± 2,75	NA	NA
Swollen joints, mean [IQR]± SD	2,97 ± 2,13	NA	NA
HAQ total scores, mean [IQR]± SD	3.56 ± 2.54 [3.97]	NA	NA
Anti RA 33 positivity, % patients	56	5	**0.005**
Anti RA33, mean [IQR]± SD IU/ml	37.99±38.68 [30.01]	18.25 ± 9.29 [16.5]	**0.031**
ESR, mean [IQR]± SD, mm/hour	41.65 ± 18.41 [40.50]	15.35 ± 4.35 [14.5]	**0.001**
CRP, mean [IQR]± SD, mg/l	18.96 ± 12.21 [19.50]	2.55 ± 1.15 [2.50]	**0.002**

† Anti RA 33: Heterogeneous Nuclear Ribonucleoprotein A2, BMI: Body Mass Index, CRP: C Reactive Protein, DAS 28: Disease activity score 28, ESR: Erythrocyte Sedimantation Rate, HAQ: Health Assesment Questionarre, NA: Not assessed.

Table 2. Comparison of rheumatolojical 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

Table 3. Demographic and clinical data 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
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

† HAQ: Health Assesment Questionarre, DMARDs: Disease-modifying antirheumatic drugs
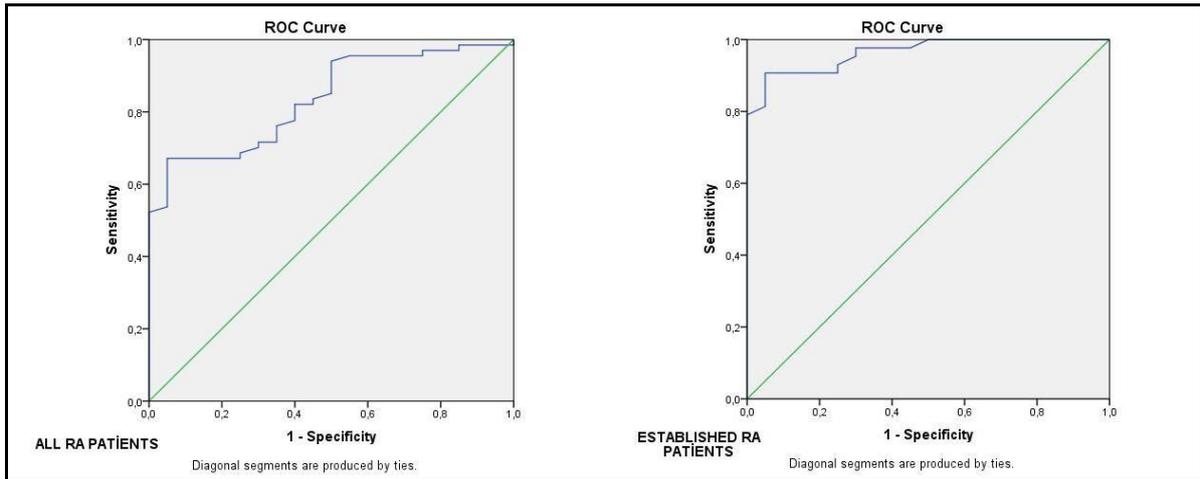
Fig, 1: Sensitivity and spesificity of Anti RA 33 in established and all RA patients.
The number of DMARDs taken was 2.80 ± 0.61 in patients negative for Anti-RA 33 but 1.58 ± 0.54 in those positive for Anti-RA 33. A strong negative correlation was evident between Anti-RA 33 titers and number of DMARDs taken (p<0.000, r= -0,766) (Figure 2).
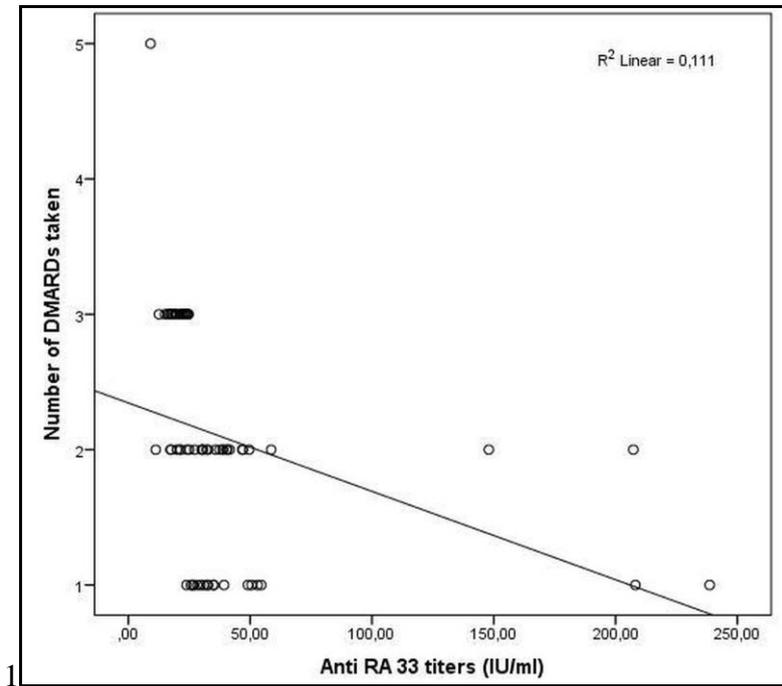

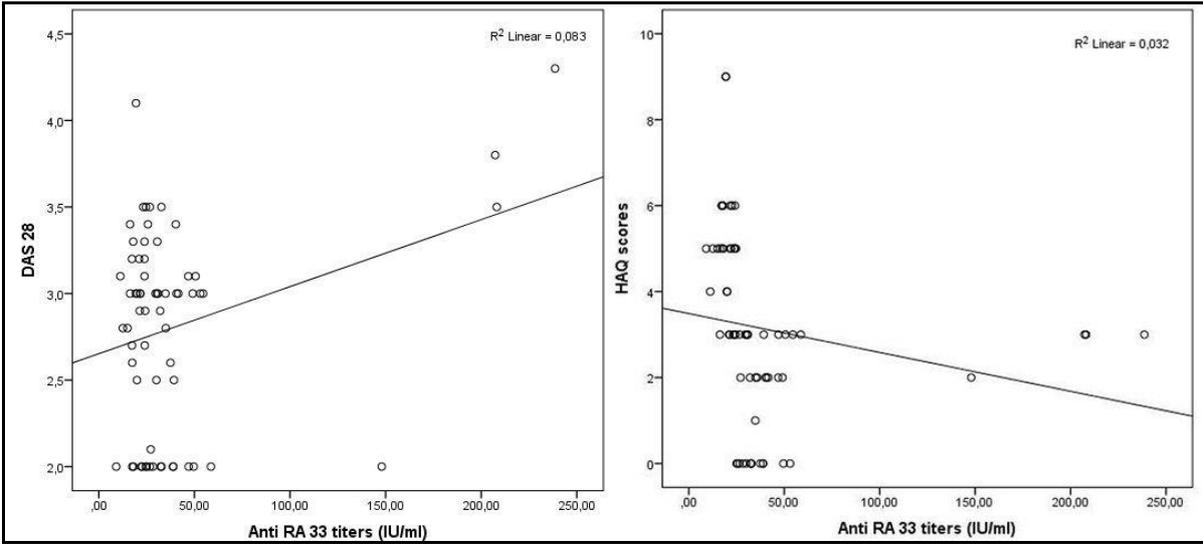Fig. 2: Relationship of anti RA 33 titers and number of DMARDs taken in study patients.

Fig. 3: Relationship of anti RA 33 titres and HAQ, DAS 28 scores.