Correlation of autoantibodies with the Disease Activity Score 28 and radiographic hand joint damage in rheumatoid arthritis patients

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a systemic chronic inflammatory disease of the joint that causes deformity or disability leading to a decreased function in RA patients. According to the 1987 American College of Rheumatology, rheumatoid factor (RF) is used as one of the diagnostic criteria because until today it is still considered as the primary autoantibody in RA although it has a lower specificity than that of anti-cyclic citrullinated peptide (anti-CCP). Besides RF and anti-CCP, anti-RA33 is another autoantibody found. The presence of the three autoantibodies in RA patient serum is important because it is the starting point of the pathogenesis of the autoimmune process in RA.

Methods: This is a cross-sectional study using consecutive sampling. Forty six subjects, all suffering from RA, were recruited for this study. All of them were tested for RF, anti-CCP, anti-RA33 titers using enzyme-linked immunosorbent assay (ELISA) method and had their hand radiograph taken to obtain the Sharp score to evaluate joint damage. During this study, 28-joint Disease Activity Score (DAS28) (4 parameters) was also evaluated using erythrocyte sedimentation rate as one of the parameters.

Results: The study found that the correlation between the three antibodies and DAS28 was not statistically significant: RF ($r = 0.200$, $p = 0.091$), anti-CCP ($r = 0.117$, $p = 0.220$), and anti-RA33 ($r = 0.126$, $p = 0.202$). There was a significant correlation between anti-CCP and the Sharp score ($r = 0.300$, $p = 0.021$). The correlation between the other two autoantibodies and the Sharp score was not statistically significant: RF ($r = 0.194$, $p = 0.098$), anti-RA33 ($r = 0.156$, $p = 0.150$).

Conclusion: There was a significant correlation between anti-CCP autoantibody and radiographic hand joint damage in RA patients so that it could be used as an indicator for occurrence of an erosive or a more severe RA.

Rheumatoid arthritis (RA) is one form of systemic chronic inflammatory rheumatic disease in which up to this day its etiology has not been fully explained yet. The course of RA disease has clinical characteristics that result in joint damage, functional disability, lower quality of life, and shorter life expectancy.[1,2] Until today, the diagnosis of RA is made based on the criteria from the 1987 American College of Rheumatology (ACR) in which its validity is now being questioned especially in regards to diagnosing early RA because the rheumatoid factor (RF) is present in only 50% of early RA and the radiographic findings showing erosion which is characteristic of early RA is found in only 13%.[3] This limitation should draw our attention because diagnosis must be made as early as possible in RA management; thereby, enabling us to predict erosive RA so that disease-modifying antirheumatic drugs could be administered as soon and aggressively as possible to slow down the process of joint damage.

Some studies reported that in the course of the RA disease, RF or anti-cyclic citrullinated peptide (anti-CCP) is correlated with the occurrence of a more erosive RA. On the other hand, the clinical feature of RA seems to be different among various ethnic groups and not all RA patients have positive RF or anti-CCP. These findings led us to a new paradigm: is RA a single disease or has it a particular subset related to genetic risk factors?[3] Also in its natural course, questions remain whether these autoantibodies of all the ethnic groups are related to joint damage in RA patients so that they could be used as the predictors of erosive RA.

The natural course of RA disease is difficult to predict-which patient has a tendency to have progressive disease with persistent inflammation and joint destruction, which patient has slow progress of disease, and which patient has fluctuating disease. It is hoped that the understanding of the correlation between RF, anti-RA33, and anti-CCP with 28-joint Disease Activity Score (DAS28) and joint damage in RA patient could be beneficial for monitoring the patient and for administering treatment more effectively.

Based on the above points, questions for this study were made: do the titers of RF, anti- RA33, and anti-CCP have positive correlation with DAS28 and joint damage in hand radiograph of RA patients?

METHODS

This is a cross sectional study using consecutive sampling. Forty six subjects, all suffering from RA, were recruited for this study. All subjects were tested for their RF titer using IMTEC-RF IgM from HUMAN, anti-CCP titer using IMTEC-CCP-Antibodies from HUMAN, anti-RA33 titer using IMTEC-RA33 antibody from HUMAN using enzyme-linked immunosorbent assay (ELISA) method and hand radiography was performed for calculation of Sharp score to evaluate joint damage. During this study evaluation of DAS28 (4 parameters) using erythrocyte sedimentation rate as one of the parameters was conducted.

RESULTS

Of 46 subjects in this study, 40 subjects (87%) were female and 6 subjects (13%) were male. The majority, 44 subjects (95.7%), were married. Although the subjects in this study were RA patients who underwent regular follow-ups at the rheumatology clinic at Cipto Mangunkusumo General Hospital, Jakarta, they were of various ethnic groups: Javanese 15 subjects (32.6%), Sundanese 12 subjects (26.1%), Batak 7 subjects (15.2%), Betawi 3 subjects (6.5%), Minang 3 subjects (6.5%), Lampung 2 subjects (4.3%), and 1 subject (2.2%) each for the following ethnic: Kalimantan, Malay, and Acehnese.

Mean age of the subjects was 48.61 years old, and if categorized in decades: 1 subject (2.2%) was in the 20–30 year old age bracket, 8 subjects (17.4%) in 31–40 year bracket, 15 subjects (32.6%) in 41–50 year bracket, 18 subjects (39.1%) in 51–60 year bracket, and 4 subjects (8.7%) in 61–70 year bracket. In this study the majority was in the 51–60 age bracket.

Disease activity evaluation using DAS28 findings were as follows: 13 subjects (28.3%) had high disease activity (DAS28 >5.1), 19 subjects (41.3%) had moderate disease activity (DAS28 3.2–5.1), 8 subjects (17.4%) had low disease activity (DAS28 2.6–3.2), and 6 subjects (13%) were in remission (DAS28 <2.6).

The outcome of Sharp score used as a dependent variable in this study was evaluated by a consultant radiologist. An intraobserver reliability analysis was conducted that showed the observer intraclass correlation coefficient values were high: joint narrowing score was 0.9848, erosion score 0.9674, and total Sharp score 0.9618. The limitations in this study did not allow the evaluation of the Sharp score to be conducted by two or three observers.

Other characteristic data such as: clinical signs of pain and swelling, visual analog scale (VAS) global health score, DAS28 score, duration of disease (months), autoantibodies level, and Sharp score based on mean and median values of the subjects are elaborated in table 1.

Table 1 Characteristics of subjects

Characteristics	Mean (SD)	Median
Age, years	48.61 (9.23)	50.0
Number of children	3.25 (2.57)	3.0
BMI, kg/m^2	24.60 (3.64)	24.25
Tender joint count	5.41 (6.28)	3.0
Swollen joint count	2.54 (3.67)	0.0
VAS global health score	17.50 (17.76)	10.0
ESR, mm/hr	58.50 (32.10)	52.50
DAS28 score	4.24 (1.37)	4.3
RF titer, IU/mL	57.13 (75.29)	22.77
Anti-CCP titer, IU/mL	828.48 (1264.74)	57.6
Anti-RA33 titer, IU/mL	49.85 (99.83)	7.65
Disease duration, months	55.24 (42.70)	39
Joint narrowing score	5.37 (6.26)	3.0
Bone erosion score	3.50 (4.70)	2.0
Total Sharp score	8.76 (9.62)	5.0

BMI, body mass index; VAS, visual analog scale; ESR, erythrocyte sedimentation rate; RA, rheumatoid arthritis; DAS28, 28-joint Disease Activity Score; RF, rheumatoid factor; anti-CCP, anti-cyclic citrullinated peptide.

Correlation of RF with DAS28 and joint damage in hand radiograph

We found that 19 subjects (41.3%) were RF negative (level <15 IU/mL) and 27 subjects (58.7%) were RF positive. Data normality test using Kolmogorov-Smirnov method found that the RF titer, DAS28, and Sharp score showed abnormal distribution so that the bivariate statistical analysis used was nonparametric statistical test. The result of Spearman's correlation test between RF titer and DAS28 showed that they were not significantly correlated ($r = 0.200$, $p = 0.091$). The test between RF titer and joint damage in hand radiograph using Sharp score also showed that they were not significantly correlated ($r = 0.194$ and $p = 0.098$).

Correlation of anti-CCP with DAS28 and joint damage in hand radiograph

The test of anti-CCP titer found that 20 subjects (43.5%) were anti-CCP negative (level ≤25 IU/mL) and 26 subjects (56.5%) were anti-CCP positive. Data normality test using Kolmogorov-Smirnov method, either for the anti-CCP titer or the Sharp score, had an abnormal distribution so that the bivariate statistical analysis used was nonparametric statistical test. The Spearman's analysis test outcome showed that the correlation between anti-CCP and DAS28 was not significant ($r = 0.117$, $p = 0.220$), but we found a significant correlation between anti-CCP and Sharp score ($r = 0.300$, $p = 0.021$). The correlation above showed that the higher the anti-CCP titer, the more hand joint damage based on the Sharp score found. The scatter diagram of the correlation between anti-CCP titer and joint damage in hand radiograph based on the Sharp score could be seen in figure 1.

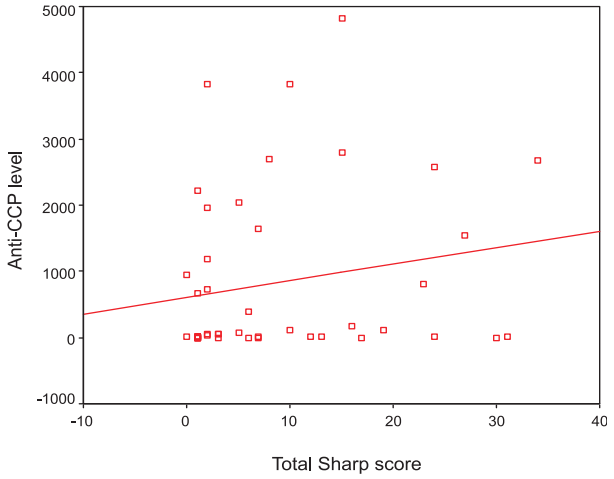

Figure 1 Scattered diagram of the correlation between anti-CCP titer and hand joint damage.

We also found no significant correlation between anti-CCP and bone erosion score both in subjects with disease duration of less than 3 years (p = 0.401) and more than 3 years (p = 0.640).

Correlation of anti-RA33 with DAS28 and joint damage in hand radiograph

The outcome of anti-RA33 titer test showed that 32 subjects (69.57%) were anti-RA33 negative (level <15 IU/mL) and 14 subjects (31.43%) were anti-RA33 positive. Data normality test using Kolmogorov-Smirnov method showed that the anti-RA33, DAS28, and Sharp score were of abnormal distribution; therefore, a nonparametric statistical test was used for the bivariate statistical analysis. The Spearman's test for the correlation between anti-RA33 titer and DAS28 showed that the correlation was not significant (r = −0.075, p = 0.311). The correlation between anti-RA33 and joint damage in hand radiograph using the Sharp score showed that the correlation was not statistically significant (r = 0.156, p = 0.150).

DISCUSSION

Rheumatoid factor has been known to be one of the autoantibodies that play a role in RA pathogenesis. Rheumatoid factor is an autoantibody against Cγ22-Cγ3 interface Fc region of the immunoglobulin molecule in which its presence in the serum is quite sensitive but not too specific. High RF titer in the serum is reported to be correlated with the severity of joint damage and extra-articular manifestations in RA.[4]

RA patients are in a state of active inflammation of the joints as a result of the differentiation of CD20-CD28+ plasma cells in the synovial fluid produced by a structure resembling the germinal centre of the lymphoid tissue. T cells play an important role in stimulating lymphocyte B cells to shift from the production of IgM RF to secretion of IgG or IgA RF to initiate the immune mechanism in RA. The presence of IgM RF is dominant in RA patient serum while IgG and IgA are dominant in synovial fluid. The differences in RF isotypes are based on the differences in the molecular shape. IgM RF is a pentamer and forms a complex with IgG, IgA RF is monomer and polymer, and IgG RF is a monomer and could form self-associated complex. IgG RF itself could form a large complex that will play a role in RA joint inflammation and IgA RF is more associated with causing bone erosion.[4-6] Knijff-Dutmer et al reported the result of RF test using the fluoroimmunoassay method that showed IgM RF was weakly correlated with the Sharp score (r = 0.19) and it seemed that IgA RF was more correlated with joint damage based on radiography.[7] Vallbracht et al reported that the RF isotype played a role in the course of RA disease—those with high IgA RF titer were more correlated with a more severe disease activity and the occurrence of joint damage especially if there was a high anti-CCP level.[8] A study conducted by De Rycke et al found that if RF is combined with the presence of shared epitope then it would be correlated with joint damage according to the radiographic score progression. The RF titer is more independent particularly it is more correlated with the occurrence of rheumatoid nodule in RA patients.[9]

The fact that rituximab treatment significantly affects only RA patients with positive RF led to a new insight that the pathogenesis of RA with positive RF is different from that with negative RF. The genetic study in a group of RF-negative RA patients found that there was no shared epitope (SE) allele such as that of positive RF.[5,6] A study conducted by Bas et al found that RA with positive IgM RF was correlated with the severity of erosion in joint damage based on the Larsen score compared to those in seronegative RA, particularly those with a disease duration of >12 years.[10] Mewar et al reported that subjects with S2 allele (K-R-A-A in position 71–74) were associated with severe or more erosive RA (p = 0.0059) and was associated with the high RF titer. Erosive RA is affected not only by RF titer level but also the presence SE allele.[11] Today we know that RA has a particular subset that is associated with SE allele that affects the severity of the course of RA disease.

This study failed to show statistically significant correlation between RF titer with DAS28 and hand joint damage. Some subjects were RF seronegative; several literatures mentioned genetic analysis of RA seronegative did not have SE allele and showed a different pathogenesis from that of RA with RF seropositive. There is an assumption that the SE allele in polymorphic genes is different in each ethnic group in Indonesia; however, whether this could affect the statistical significance had not been researched in this study. This study used a cross-sectional method so that the titer level obtained was only at one point in time and only measured the IgM RF which could also affect the statistical significance. Further studies comparing the seropositive RA group with seronegative RA using other RF isotype and using the cohort method is recommended.

In RA, we could find a variety of antibodies against structures produced by keratinized epithelial cells which include antikeratine antibody, antiperinuclear factor, and antifilagrine antibody. The target molecules of these antibodies among others are filagrine, vimentine, and other peptides. Peptidylarginine deiminase enzyme is in charge of converting the arginine epitope to citrulline that has autoantigenic characteristic in which individuals who have a genetic risk

for RA expresses the citrulline protein fragment through the MHC class II T cell molecule. Next, B cell response to citrulline antigen forms (anti-CCP) autoantibodies that later stimulates the chronic inflammatory process that is the basis of RA pathogenesis.[12] Various studies reported that anti-CCP has higher specifity, which is between 94–96%, compared to that of RF. One study conducted by Vallbract et al reported that although the three RF isotopes were negative in RA patients, anti-CCP could be found positive in 34.4% of them.[8] Various studies found that anti-CCP has high sensitivity and specificity so that if RF is negative, anti-CCP test should be next carried out.

Some cohort studies found that anti-CCP in the serum is found long before RA is diagnosed; therefore, anti-CCP is considered as predictor for the occurrence of RA in the future in patients with polyarthritis. For diagnosing RA, anti-CCP is 20% more sensitive than RF. It is proven that RA patients with positive anti-CCP have the associated genetic risk factors, among others: human leukocyte antigen shared epitope alleles (HLA-SE), protein tyrosine phosphatase nonreceptor type 22 (PTPN22), complement component 5-TNF receptor-associated factor 1 (C5-TRAF1), and TNFα-induced protein 3-oligodendrocyte lineage transcription factor 3 (TNFAIP3-OLIG3). The presence of HLA-DRB1*0401 and HLA-DRB1*0404 causes increased affinity of a peptide to bind with HLA-DRB1 during the conversion of arginine to citrulline. In the next stage, the CD4+ T cell is activated which stimulates B cell activation resulting in the production of anti-CCP and at the end the formation of immune complex that triggers the inflammatory process through the upregulation of the proinflammatory cytokine. This evidence shows that anti-CCP plays a dominant role in the development of chronic inflammation of the joints in RA. RA patients with a continuously high positive anti-CCP titer and presence of SE allele will likely have joint damage.[4-6,12]

In this study, the correlation between anti-CCP and DAS28 was not statistically significant, probably because the anti-CCP titer at one point of time was not enough to prove its correlation with RA activity. The correlation between anti-CCP and joint damage according to the Sharp score was significantly positive with a correlation coefficient of 0.30. This outcome is in line with the subjects of this study who were nonsmokers. A study by Mattey et al reported that smoking will elevate anti-CCP titer and this also occurs to those who have HLA-DRB1 SE.[13]

Result of other studies supported our study, among others: Avouac et al that reported high specificity of anti-CCP of 95–96% in RA and Bas et al, Meyer et al, and Bukhari et al that reported anti-CCP had high specificity (96%) and positive subjects were strongly correlated with the occurrence of erosive RA whose severity is in line with the duration of RA disease.[10,14,15] Forslind et al and Syversen et al reported that anti-CCP was correlated with radiographic joint damage and could be used as an independent predictor for progression and radiographic RA damage so that it could be used as an indicator for the rheumatologist in clinical practice to determine whether a more aggressive treatment is needed.[16,17] According to a study conducted by Lee et al, 81% of RA patients with erosion showed reactive anti-CCP. Liao et al suggested revision of the RA diagnostic criteria of the 1987 ACR by including anti-CCP as one of the criteria to replace rheumatoid nodule. They found that the sensitivity was higher at 55% and specificity remained high at 91%.[12,18-21]

This study could not provide enough evidence to support the correlation between anti-RA33 and DAS28 nor joint damage of the RA hand according to Sharp score. The anti-RA33 titer taken was at one point of time and 50% of the subjects were negative resulting in an insignificant correlation in the statistical analysis. Despite this fact, Mediwake et al reported an optimistic result in which anti-RA33 could be used to differentiate erosive RA from erosive SLE. A further study is needed to prove this fact.[22]

CONCLUSION

The anti-CCP autoantibody is significantly correlated with joint damage in hand radiograph of RA patients; therefore, it could be used as an indicator for the occurrence of an erosive or a more severe RA.

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