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ORIGINAL ARTICLE

Diagnostic Performances of Anti-Cyclic Citrullinated Peptide Antibodies type IgM, IgA and IgG in Syrian Patients with Rheumatoid Arthritis

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SUMMARY

Background: **To determine the diagnostic performances of anti-cyclic citrullinated peptide antibodies (anti-CCP) type IgM, IgA and IgG and rheumatoid factor (RF) in Syrian patients with rheumatoid arthritis.**
Methods: **64 patients with rheumatoid arthritis were included in our study. Anti-CCP IgM, IgA and IgG and rheumatoid factor (RF) were detected using ELISA.**
Blood samples were collected from patients with definite rheumatoid arthritis according to (ACR) criteria in Al Mwasaa University Hospital and Al Assad University Hospital, Damascus, Syria, from December 2007 to December 2008.
Results: **The sensitivity of anti-CCP IgG was 71.9 % and specificity was 100 %, Whereas the sensitivity of anti-CCP IgM was 70.3 % and specificity was 64 %, the sensitivity of anti-CCP IgA was 43.75 % and specificity was 100 %, RF IgM showed a sensitivity of 70.3 % and a specificity of 96 %, and anti-CCP IgG prevalence in patients with negative RF was 31.6 %.**
All tests showed no correlation with gender in RA patients.
Conclusions: **This study demonstrates that anti-CCP IgG is a highly specific marker for RA and has diagnostic value especially in RF negative patients.**
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KEY WORDS

Rheumatoid Arthritis: RA
Anti-cyclic citrullinated peptide antibodies: anti-CCP
Rheumatoid Factor: RF
peptidylarginine deiminase : PAD

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease affecting about 1 percent of the general population worldwide (1). The etiology of RA remains a mystery, but both environmental and genetic factors contribute in the development of the disease (2). Although RA exhibits a variety of extra-articular manifestations (e.g. vasculitis), the synovium is the primary site of pathology in RA (1). RA, a chronic and progressive disease, leads to gradual destruction and functional damage of joints. The severity and rapidity of disease differs from patient to patient (3).

Autoantibodies in RA :
Like the other systemic autoimmune diseases, RA is characterized by the occurrence of a large number of autoantibodies against a variety of antigens, but most of these autoantibodies lack the specificity or the sensitivity for RA(4). Rheumatoid factors (RF) are the first autoantibodies to have been discovered, and it still is the traditional serological test for the diagnosis of RA. Generally, RF is associated with rheumatoid arthritis (RA) but it can be found in several diseases like other rheumatic diseases, acute and chronic inflammatory diseases, and viral infections, as well as in normal individuals (especially the elderly) (4-5).
Recently, new autoantibodies have been identified in

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the sera of patients with RA. These new autoantibodies are specific for epitopes containing the unusual amino acid citrulline that is generated by post-translational deimination of arginyl residues by the enzyme peptidyl arginine deiminase (PAD) (Figure 1) (6).
Citrulline-containing peptides were used in the assay but the sensitivity was low (6).
To improve the assay, cyclic variants of these peptides were developed and used as antigen in the first generation assay of these autoantibodies and were called anti-cyclic citrullinated peptides antibodies (anti-CCP) (6-7).
Screening a library of citrullinated peptides with a pool of RA sera led to the development of the second generation CCP test (CCP2) (2,4). The CCP2 test is characterised by high sensitivity and specificity to RA as has been showen in many studies (4).
Both the CCP1 and CCP2 tests detect autoantibodies against citrullinated peptides type IgG. Recently, new tests were developed to determine anti-CCP type IgM and type IgA but have not yet been studied.
We determined the diagnostic performances of anti-CCP type IgG, type IgM and type IgA and compared those with the diagnostic performance of RF.

MATERIALS AND METHODS

Serum samples were obtained from 64 patients (46 women and 18 men) at their presentation to the rheumatology clinic in Al Mwasaa University Hospital and Al Assad University Hospital, Damascus, Syria, from January 2007 to January 2008. Patients were diagnosed by a rheumatologist as RA according to the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) criteria (16).
Controls included 25 apparently healthy subjects (13 women and 12 men) and were chosen from the Syrian population who were donating their blood at the blood bank of Damascus University.
All controls were checked by the internal doctor of the blood bank. The study protocol was approved by the ethics committee of the University Hospital, Damascus, Syria, and informed consent was obtained from all patients.

Data measurement and analysis :
CCP measurement: Anti-CCP type IgG was determined by an enzyme linked immunosorbent assay (ELISA) using a commercial anti-CCP2 assay provided by the EUROIMMUN Corp.
Anti-CCP type IgA was determined by an enzyme linked immunosorbent assay (ELISA) using a Citrullinated Protein Antibodies IgA ELISA Kit provided by the GENESIS *Diagnostics* Corp (the components of the kit are research use only).
Anti-CCP type IgM was determined by an enzyme linked immunosorbent assay (ELISA) using a Citrullinated Protein Antibodies IgM ELISA Kit provided by the GENESIS *Diagnostics* Corp (the components of the kit are research use only).
Rheumatoid factor measurement : RF IgM was determined by ELISA using an IMTEC-RF IgM test provided by the IMTEC-HUMAN Corp.
All of the assays used were performed according to the manufacturers' instructions.

Statistical analysis. The data were subjected to statistical evaluation, using the Statistical Program for Social Science (SPSS version 14) for Windows. Results were expressed as mean ± SD. Correlations between variables were assessed using Pearson's correlation coefficient. T-test was performed to determine differences between variables. A p-value of <0.05 was considered statistically significant. Sensitivity and specificity were calculated. The optimal cut-off for all tests was determined by a receiver operating characteristics (ROC) curve.
(The ROC curve is a graphical plot of the sensitivity vs. (1 − specificity) for a binary classifier system as its discrimination threshold is varied).

RESULTS

Anti-CCP IgG, anti-CCP IgM, anti-CCP IgA, and RF IgM showed significant differences in the mean value between RA patients and controls (Table 1, Figure 2).
We determined sensitivity and specificity of anti-CCP IgG, anti-CCP IgM, anti-CCP IgA, and RF IgM at different test cut off values by generating ROC curves (95 % Confidence Interval).
Anti-CCP IgG. ROC curve analysis found the optimal cut off value of anti-CCP IgG to be (1 RU/mL), at which anti-CCP IgG showed 71.9 % of sensitivity and 100 % of specificity (Figure 3). All controls were negative at this cut off value.
Anti-CCP IgG. Anti-CCP IgG was positive in 6/19 (31.6 %) patients with negative RF.
Anti-CCP IgM. The optimal cut off value for anti-CCP IgM was (22 U/mL). Anti-CCP IgM showed 70.3 % sensitivity and 64 % specificity (Figure 4).
Anti-CCP IgA. The optimal cut off value for anti-CCP IgA was (1 U/mL), Anti-CCP IgA showed 43.75 % sensitivity and 100 % specificity (Figure 5).
RF IgM. The optimal cut off value for RF IgM was (26.5 U/mL). RF IgM showed 70.3 % sensitivity and 96 % specificity (Figure 6).
In our study the male:female ratio was 1:2.6 .
The study showed no significant difference in the mean value between men and women for anti-CCP IgG, anti-CCP IgM, anti-CCP IgA, and RF IgM (Table 2, Figure 7).

Table 1. Test results at the optimal cut off value for anti-CCP IgG, anti-CCP IgM, anti-CCP IgA, and RF IgM in RA patients and controls.

AUTOANTIBODY	RA mean ± SD	CONTROLS mean ± SD	p-value
anti-CCP IgG	54.6 ± 75.1	0.27 ± 0.18	Less than 0.0001
anti-CCP IgM	44 ± 39.2	22.5 ± 11.2	Less than 0.0001
anti-CCP IgA	12.6 ± 34.8	0.11 ± 0.18	Less than 0.0001
RF IgM	103.8 ± 91.7	7.5 ± 9.1	Less than 0.0001

Table 2. Serum values of anti-CCP IgG, anti-CCP IgM, anti-CCP IgA, and RF IgM in men and women RA patients.

AUTOANTIBODY	Men mean ± SD	Women mean ± SD	p-value
anti-CCP IgG	42.7 ± 65.3	58.9 ± 78.5	P>0.05
anti-CCP IgM	48.6 ± 46.9	42.4 ± 36.5	P>0.05
anti-CCP IgA	15.2 ± 37.2	11.7 ± 34.2	P>0.05
RF IgM	122.7 ± 107.8	97 ± 85.4	P>0.05

Table 3. Diagnostic value of CCP2 Test.

Study	Reference	Sensitivity (%)	Specificity (%)
Van Venrooij	8	82	98
Vasishta	9	79	97
Pinheiro	10	80	98
Lee	11	66	90
Suzuki	12	88	89
Dubucquoi	13	65	96
Grootenboer-Mignot	14	63	91
Vallbracht	15	64	97

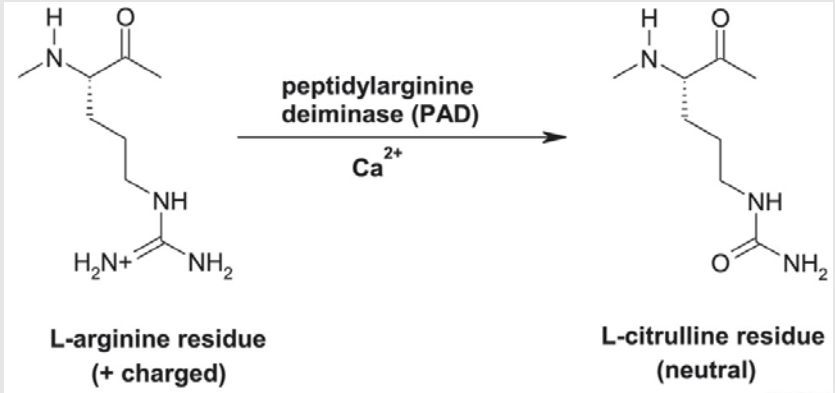

Figure 1. Citrullination (deimination) of peptidylarginine by PAD.
Citrullination is the posttranslational modification of arginine amino acid residues into citrulline residues. This reaction is catalyzed by peptidylarginine deiminase (PAD) enzymes.

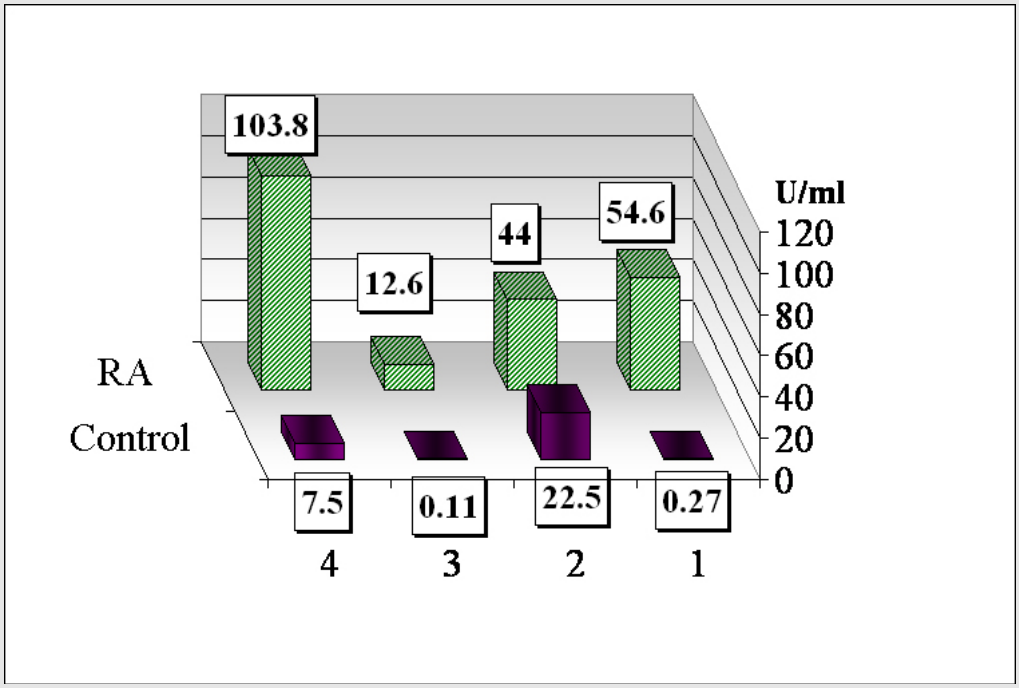

Figure 2. Mean serum levels of 1- anti-CCP IgG,2- anti-CCP IgM,3- anti-CCP IgA, 4- RF IgM in RA patients and controls.

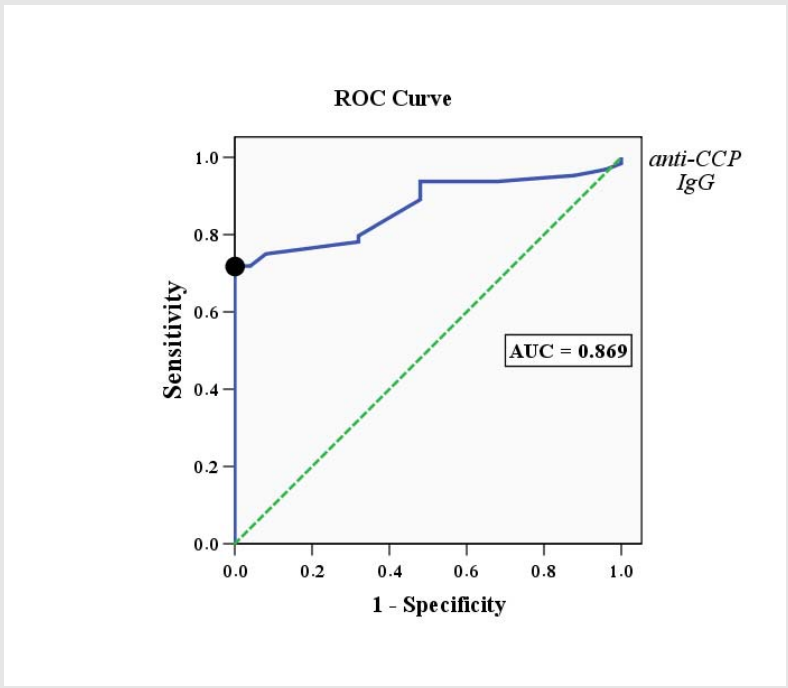

Figure 3. Receiver operating characteristic curve of anti-CCP IgG.
The *black dot* at the upper left is at the titer of 1 RU/mL, with sensitivity and specificity of 71.9 % and 100 %, respectively.

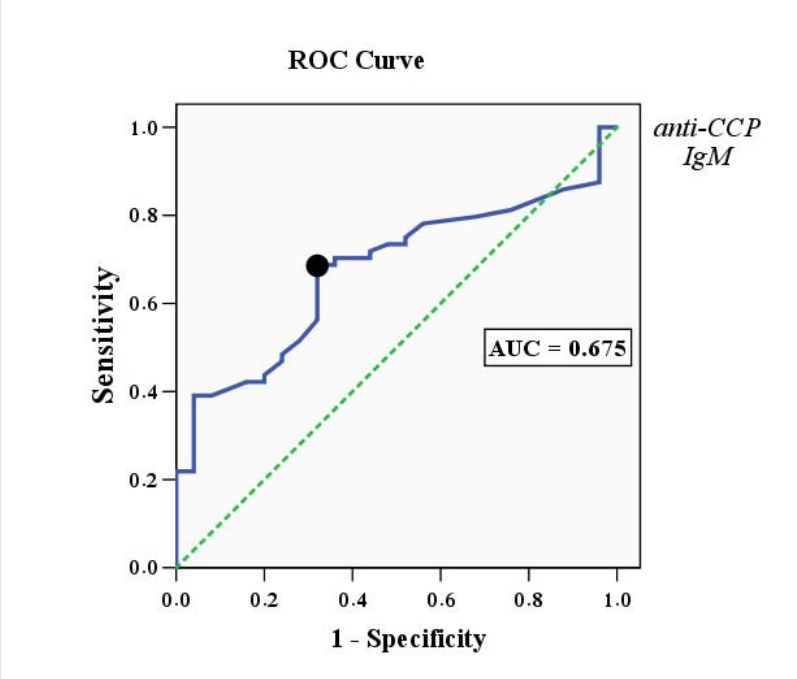

Figure 4. Receiver operating characteristic curve of anti-CCP IgM.
The *black dot* at the upper left is at the titer of 22 U/mL, with sensitivity and specificity of 70.3 % and 64 %, respectively.

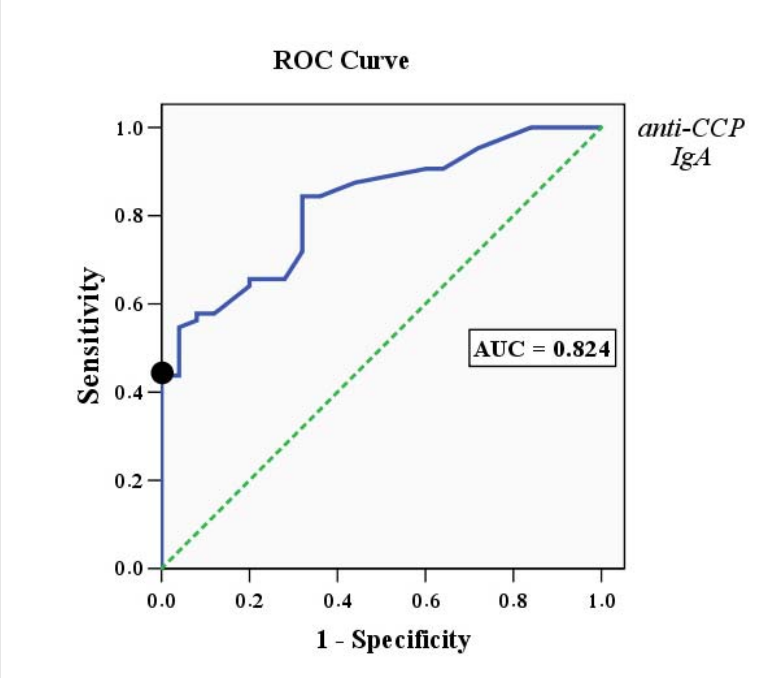

Figure 5. Receiver operating characteristic curve of anti-CCP IgA.
The *black dot* at the upper left is at the titer of 1 U/mL, with sensitivity and specificity of 43.75 % and 100 %, respectively.

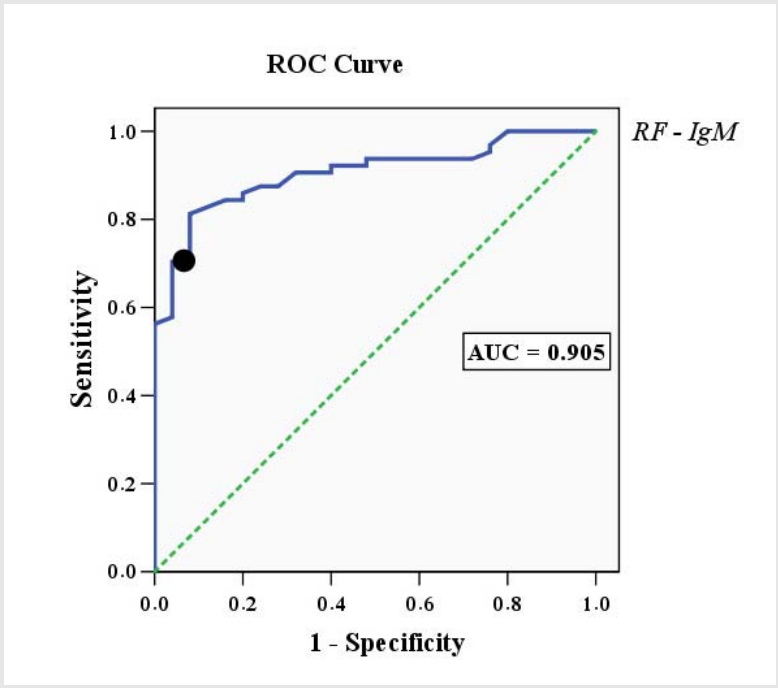

Figure 6. Receiver operating characteristic curve of RF - IgM .
The *black dot* at the upper left is at the titer of 26.5 U/mL, with sensitivity and specificity of 70.3 % and 96 %, respectively.

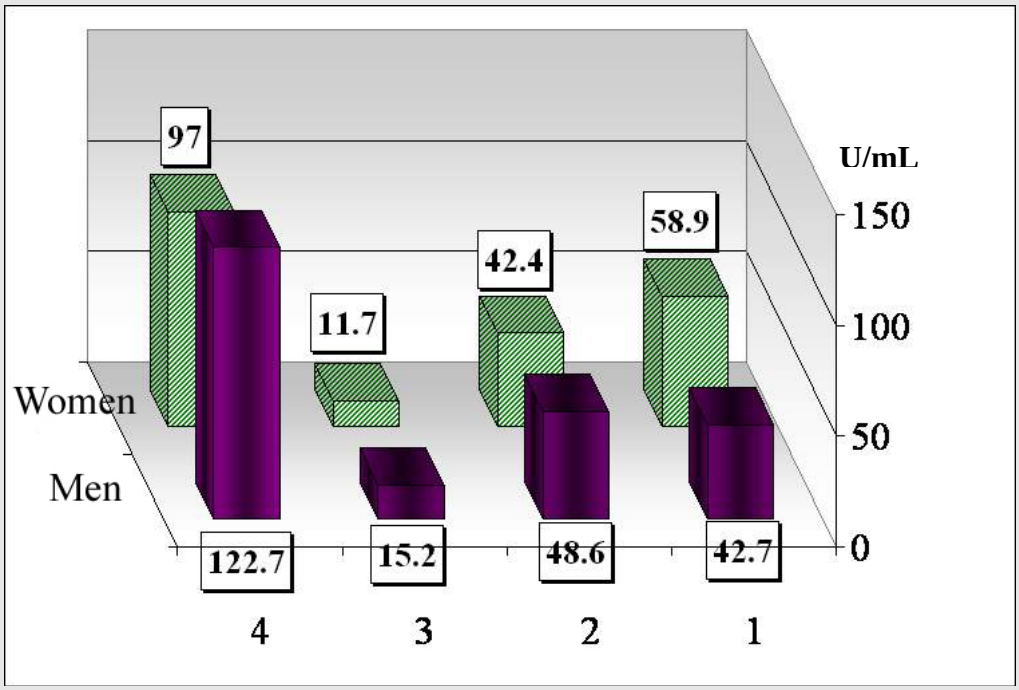

Figure 7. Mean serum levels of 1- anti-CCP IgG,2- anti-CCP IgM,3- anti-CCP IgA, 4- RF IgM in women and men.

DISCUSSION

In our study we evaluated the diagnostic performances of anti-CCP type IgG, type IgM, type IgA and RF type IgM by the ELISA method.

At the optimal cut off values, 46 of 64 RA patients (71.9 %) were positive for anti-CCP IgG, whereas none of the 25 controls showed a positive reaction (specificity: 100 %). The number of anti-CCP IgM positives was 45 of 64 RA patients (70.3 %), 9 of 25 controls (specificity: 64 %). The number of anti-CCP IgA positives was 28 of 64 RA patients (43.75 %), 0 of 25 controls (specificity: 100 %), and for RF IgM 45 of 64 RA patients (70.3 %) were positive and one of the 25 controls showed positivity (specificity: 96 %).

Sensitivities were close between anti-CCP IgG, anti-CCP IgM, and RF IgM but were low for anti-CCP IgA. The anti-CCP IgG assay had higher specificity than other assays.

For anti-CCP IgG, our results were similar to several studies (Vasishta et al, Dubucquoi et al, and Vallbracht et al. (9,13,15) (Table 3). The results for RF IgM, especially the sensitivity, were similar to the study of Suzuki et al and Goldbach-Mansky et al (12,17).

In RA patients, the male:female ratio was 1:2.6, the mean values of anti-CCP IgG, anti-CCP IgM, anti-CCP IgA, and RF IgM between men and women showed no significant difference. These results confirm those obtained by Choi et al. (18).

No data are currently available on the diagnostic performances of anti-CCP type IgM or IgA. The determination of the diagnostic performances of these antibodies need further studies in a larger cohort of patients.

As this is a sectional study, the sample size was small and hence the results should be interpreted with caution.

CONCLUSION

The data presented herein show that anti-CCP IgG is the most specific marker for RA and provides an additional diagnostic tool especially in RF negative patients.

We conclude that anti-CCP IgG assay is very useful for the diagnosis of RA.

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