Anti-CCP antibodies, rheumatoid factors and anti-keratin antibodies: clinical value in established rheumatoid arthritis

Mohamed Amri*, Imen Sfar*, Hajer Skhiri***, Tarek Dhaouadi*, Noura Bel Hadj*, Leila Abdelmoula**, Rafik Zouari**, Khaled Ayed*, Yousr Gorgi*.

*Departments of Rheumatology** and Immunology*. Charles Nicolle Hospital. Public Health Institute***. Tunis. Tunisia. Université Tunis El Manar*

M. Amri, I. Sfar, H. Skhiri, T. Dhaouadi, N. Bel Hadj, L. Abdelmoula, R. Zouari, K. Ayed, Y. Gorgi.

Anticorps anti-CCP, facteurs rhumatoïdes et anticorps anti-kératine : valeur clinique dans la polyarthrite rhumatoïde avérée.

LA TUNISIE MEDICALE - 2011 ; Vol 89 (n°03) : 231 - 235

M. Amri, I. Sfar, H. Skhiri, T. Dhaouadi, N. Bel Hadj, L. Abdelmoula, R. Zouari, K. Ayed, Y. Gorgi.

Anti-CCP antibodies, rheumatoid factors and anti-keratin antibodies: clinical value in established rheumatoid arthritis.

LA TUNISIE MEDICALE - 2011 ; Vol 89 (n°03) : 231 - 235

RÉSUMÉ

Prérequis : Il est bien établi que dans les formes débutantes depolyarthrite rhumatoïde, les anticorps anti-CCP ont une meilleure valeur diagnostique que les facteurs rhumatoïdes et les anticorps anti-kératine. Néanmoins, leur rôle est moins bien connu chez les patients ayant une polyarthrite rhumatoïde avérée ou à longue durée d'évolution.

But : Evaluer et comparer les performances diagnostiques des anti-CCP, des anti-kératine, des facteurs rhumatoïdes IgM et IgA dans la polyarthrite rhumatoïde avérée.

Méthodes : Dans une étude transversale, nous avons recherché ces autoanticorps chez 90 patients ayant une polyarthrite rhumatoïde avérée et chez un groupe contrôle de 100 sujets. En outre, nous avons étudié l'association de ces marqueurs avec l'activité et la sévérité de la maladie. La sensibilité et la spécificité ont été calculées pour chacun des quatre tests, en utilisant le diagnostic clinique comme « gold standard ».

Résultats : Les anti-CCP et le facteur rhumatoïde IgM avaient la meilleure valeur diagnostique. Aucun des anticorps n'avait de relation significative avec le score d'activité de la maladie (DAS28). Après ajustement par régression linéaire multiple, les anti-CCP étaient les seuls marqueurs associés à la forme érosive de la maladie.

Conclusion : Dans la polyarthrite rhumatoïde à longue durée d'évolution, les anti-CCP et le facteur rhumatoïde IgM ont des valeurs diagnostiques similaires. Toutefois les anti-CCP sont utiles chez les patients séronégatifs. Il s'agit, en outre de marqueurs fiables d'une maladie sévère et érosive.

Mots-clés

Polyarthrite rhumatoïde, Anti-CCP, facteurs rhumatoïdes, anticorps anti-kératine.

SUMMARY

Background : It is well documented that in early rheumatoid arthritis, anti-CCP antibodies have better diagnostic value than rheumatoid factors and anti-keratin antibodies. However, their role is less well defined in patients with established or long duration disease.

Aim : To evaluate and to compare diagnostic performances of anti-CCP, anti-keratin, IgM and IgA rheumatoid factors in established rheumatoid arthritis.

Methods : In a cross-sectional study, 90 patients with established rheumatoid arthritis and 100 controls were tested for these autoantibodies. The association of these markers with disease activity and severity was investigated. The sensitivity and specificity were calculated for each of four tests, using the clinical diagnosis as the gold standard.

Results : The anti-CCP and IgM rheumatoid factor exhibited the best diagnostic value. None of the tested antibodies had any significant association with the disease activity score (DAS28). After adjustment by multiple linear regression, only anti-CCP positivity was found to be significantly associated with erosive disease.

Conclusion : In long duration rheumatoid arthritis, anti-CCP and IgM rheumatoid factor have similar diagnostic value. However anti-CCP are useful in seronegative patients. They are also a reliable marker of severe erosive disease.

Key-words

Rheumatoid arthritis, Anti-CCP, Rheumatoid factors, Anti-keratin antibodies.

Rheumatoid arthritis (RA) is a chronic progressive inflammatory disease, affecting approximately 1% of the world's population (1). The diagnosis is basically clinical. However, serological tests have an important supportive value. A good serological marker is needed, either at disease onset or during follow-up. Indeed, serological markers can help clinicians to predict prognosis and to indicate more aggressive treatment. Furthermore, in some patients with established inflammatory polyarthritis, an initial diagnosis of RA may be revised later (2).

Rheumatoid factor (RF) is the immunologic hallmark of RA. Its positivity is one of the American College of Rheumatology (ACR) classification criteria for RA (3). The conventional agglutination and immunoturbidimetric techniques measure principally IgM class RF. The specificity and sensitivity of RF have been improved by the development of enzyme-linked immunosorbent assay (ELISA), which allows measurement of RF belonging to all the major immunoglobulin classes (IgM, IgG and IgA) (4). Anti-keratin antibodies (AKA) represent another diagnostic marker for seropositive rheumatoid polyarthritis and seronegative ones (5). Nevertheless, AKA have never been widely used as markers for rheumatoid arthritis because of the subjective immunofluorescence technique and the problematic inter-laboratory standardization. In the last few years, the anti-cyclic citrullinated peptide antibodies (anti-CCP) assay has gained interest in the rheumatology community because it is reported to be the best diagnostic tool and to have the highest prognostic value (4,6,7). This superiority has been established mainly in the early course of RA. Data on anti-CCP role in established and long duration RA is limited.

The primary purpose of present cross-sectional study was to compare the diagnostic performances of anti-CCP, AKA, IgM and IgA RF in established RA. A further aim was to assess the association of these tests with the disease severity and activity.

METHODS

Ninety patients (70 women and 20 men; mean age 51.9 ± 13.1 years) meeting the ACR classification criteria for RA, were included in the study. They were recruited from the rheumatology department of Charles Nicolle Hospital between January and June 2006. Age, sex, disease duration, clinical characteristics and basic laboratory tests were recorded for each patient. Disease activity was evaluated using the DAS28 score (8). Radiological damages were evaluated by an anteroposterior view X-rays of wrists and hands and by bone X rays densitometry. One hundred healthy blood donors served as a control group.

Anti-CCP were detected by an enzyme linked immunsorbent assay (ELISA) using a second generation commercial assay (Euroimmun®, Lubeck, Germany) according to the manufacturer's instructions. AKA were examined by indirect immunofluorescence using frozen sections of rat oesophagus. Serum samples were diluted 1:5 in phosphate buffered saline (PBS). Only an intensive laminar fluorescence of the stratum corneum was regarded as positive. RF of IgA and IgM classes were detected by home-made ELISA. Results were expressed in U/ml. The cut off value was 16 U/ml for both isotypes.

Data management and analysis were carried out using Stata 9. The sensitivity and specificity were calculated for each of four tests, using the clinical diagnosis as the gold standard. A receiver operating characteristic (ROC) analysis was performed to compare sensitivities and specificities and a Chi-square test was used for comparing areas under curves. We used Student's t-test for comparisons of means and Chi-square (Pearson and McNemar) for comparisons of percentages. Fisher's exact test was applied when Chi-square test was invalid. The concordance between the tests was assessed by coefficient kappa (κ). A multiple linear regression was performed to estimate the adjusted association between "bone damages" and the studied autoantibodies. A p value less than 0.05 was considered statistically significant.

RESULTS

Demographic, clinical and laboratory features of the study patients are summarized in Table 1.

Table 1 : Demographic and clinical characteristics of the study patients

Sex (n, %)		
	Male	70 (77.8%)
	Female	20 (22.2%)
Age (mean, SD) (years)		51.9 ± 13.1
Extrarticular features		13 (14.4%)
Disease duration (years)		
	<5 years	22 (24.4%)
	5-10 years	23 (25.6%)
	≥10 years	45 (50%)
Radiological damage		
	Absence	62 (68.9%)
	Presence	28 (31.1%)
Tender or swollen joint		
	Normal joint	59 (65.6%)
		31 (34.4%)
Erythrocyte sedimentation rate		74 (82.2%)
Accelerated	Normal	16 (17.8%)
	IgM RF (+)	59 (65.6%)
	IgA RF (+)	49 (54.4%)
	Anti-CCP (+)	64 (71.1%)
	AKA (+)	11 (12.2%)

n: number, RF : rheumatoid factor.

Among the 90 patients with RA, 64 (71.1%) had anti-CCP antibodies, 59 (65.6%) had IgM RF, 49 (54.4%) had IgA RF and 11 (12.2%) had AKA. Positivity of all tested serological markers was unaffected by age, gender or extrarticular features. A low frequency of all four antibodies was observed in the healthy control group. Hence, all the performed tests had high

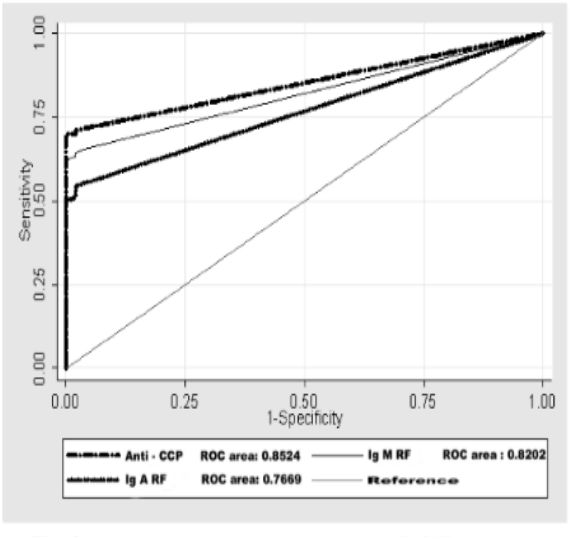
specificity exceeding 98% (Table 2). Sensitivity was highest for anti-CCP, followed by IgM RF and IgA RF. The difference between IgM RF and anti-CCP was not significant, and both were significantly better than IgA RF and AKA. The combined use of two autoantibodies resulted in higher sensitivities. The highest sensitivity (80%) was achieved by the combination of anti-CCP and IgM RF tests. Furthermore, a moderate concordance between the anti-CCP and the IgM RF titers was observed (κ: 0.46, p<0.001). Among 26 anti-CCP-negative patients, 8 (30.7%) were IgM RF-positive and among 31 IgM RF-negative patients, 7 (22.5%) were anti-CCP-positive.

Table 2 : Sensitivity and specificity of AKA, anti-CCP and RF

Serological marker	Sensitivity (CI 95%) n=90	Specificity (CI 95%) n=100
IgM RF	65.6 (54.7-75.1)	98.0 (93.0-99.8)
IgA RF	54.4 (43.6-64.9)	99.0 (94.6-99.9)
Anti-CCP	71.1 (60.5-79.9)	98.0 (93.0-99.8)
AKA	12.2 (6.6-21.2)	100.0 (96.4-100.0)
IgM RF +Anti-CCP	56.7 (45.8-66.9)	100.0 (96.4-100.0)
IgA RF +Anti-CCP	50.0 (39.4-60.6)	100.0 (96.4-100.0)
AKA+ Anti-CCP	5.6 (1.8-12.5)	100.0 (96.4-100.0)

For further comparisons of the diagnostic value of each assay, we did an ROC analysis (fig 1) and calculated the area under the curve (AUC). The AUC was best for anti-CCP, at 0.85 and for IgM RF at 0.82. The values for IgA RF and AKA were 0.76 and 0.56, respectively. Therefore, anti-CCP and IgM RF exhibited comparable diagnostic values, which were better than those of IgA RF and AKA.

Figure 1 : Receiver operating characteristic (ROC) curves



Our patients were divided according to disease duration (0-5 years, 5-10 years, more than 10 years). The patients hada median disease duration of 11 years. Neither anti-CCP nor RF positivity had any significant relationship with disease duration (Table 3).

Disease activity, as defined by the DAS28 score, was low in 8 patients (8.9%), moderate in 22 (24.4%) patients and high in 60 (66.7%) patients. No association was found between tested autoantibodies and disease activity. However, anti-CCP followed by IgM RF were more prevalent than IgA RF and AKA in the group with high disease activity.

Table 3 : Correlation between clinical characteristics and tested autoantibodies

	Anti-CCP(+) n=64	IgA RF(+) n=49	IgM RF(+) n=59	AKA(+) n=11
		41 (58.6%)	49 (70%)	8 (11.4%)
Sex : F (n=70)	51 (72.9%)	8 (40%)	10 (50%)	3 (15%)
M (n=20)	13 (65%)			
Duration		12 (54.6%)	13 (59.1%)	(22.7%)
<5 yars (n=22)	16 (72.7%)	12 (52.2%)	15 (65.2%)	4 (17.4%)
5-10 yars (n=23)	16 (69.6%)	25 (55.6%)	31 (68.9%)	2 (4.4%)
≥10 yars (n=45)	32 (71.1%)	8 (61.5%)	7 (53.9%)	1 (7.7%)
Subcutaneous nodules (n=13)	9(69.2%)			
DAS28		4 (50.0%)	5 (62.5%)	2 (25%)
<3.2 (n=8)	5 (62.5%)	12 (54.6%)	14 (63.6%)	4 (18.2%)
3.2-5.1 (n=22)	18 (81.8%)	33 (55.0%)	40 (66.7%)	5 (8.3%)
>5.1 (n=60)	41 (68.3%)			
Radiological damage				
Absence (n=62)	37 (59.7%)	28 (45.2%)	36 (58.1%)	10(16.1%)
Presence (n=28)	27 (96.4%)	21 (75%)	23 (82.1%)	1 (3.6%)
Tender or swollen joint (n=59)	53 (89.8%)	37 (62.7%)	45 (76.3%)	4 (6.8%)
Normal joint (n=31)	11 (35.5%)	12 (38.7%)	14 (45.2%)	7 (22.6)
Accelerated ESR (n=74)	54 (73 %)	43 (58.1%)	52 (70.3%)	8 (10.8%)

Erythrocyte sedimentation rate (ESR), joint tenderness and swelling are usually used for assessing disease activity. A statistically significant correlation with joint tenderness and swelling was found for anti-CCP ($p<0.001$), followed by IgM RF ($p<0.01$), IgA RF ($p<0.05$) and AKA ($p<0.05$). Autoantibodies positivity was also related to ESR, but the association was significant only for IgM RF.

None of the antibodies or combination of antibodies had any significant association with the presence of extrarticular features. Nevertheless, subcutaneous nodules were more frequent in patients with positive anti-CCP or RF.

Except for one, all patients with radiological lesions were positive for anti-CCP antibodies. Radiological lesions were also associated with IgM RF and IgA RF. After adjustment by multiple linear regression, only anti-CCP positivity was found to be significantly associated with erosive disease.

DISCUSSION

Anti-CCP antibodies have shown high specificity and relatively high sensitivity for RA diagnosis, in several cohorts of patients with early arthritis (9). Unlike early RA, data on established RA are rare. In our study, anti-CCP had a sensitivity of 71.1% and a specificity of 98%. Somewhat comparable results were found in other studies, which have reported sensitivities of 66-73% and specificities of 90-98% (2, 4, 10). Similarly, our findings in terms of IgA and IgM RF sensitivities were in the range reported by other studies (4, 7). Thus, anti-CCP and RF performance characteristics do not seem to differ significantly between early and established disease.

In agreement with other studies,anti-CCP provided the best combination of sensitivity and specificity for detecting RA. However, the difference between IgM RF and anti-CCP was less significant than in studies with short-duration cohorts (4, 6, 11). Alexiou et al (11) demonstrated an additional diagnostic value of anti-CCP in RF-negative patients. In deed, they established that 34.9 % of IgM RF-negative patients had anti-CCP antibodies. These results confirm some previous reports (4, 6, 12). In all these studies, more than the third of IgM RF-negative patients had anti-CCP antibodies. In our study, the percentage was lower (22.5%). As in RA, anti-CCP level often arises earlier than RF (13,14), the longer disease duration of our patients may explain the inferior diagnostic advantage of anti-CCP.

The diagnostic sensitivity of AKA in our RA patients was 12.2%, which is unexpectedly lower than reported in most studies (15, 16, 17). These antibodies have sensitivity varying from 40% to 70%, but they are known to be associated with disease severity and functional disability (16, 17). The low frequency of AKA in our study population could be attributed to the preponderance of mild and moderate forms of RA.

ELISA method allowed us to examine the concordance between autoantibodies levels. There was a significant correlation between the anti-CCP and the IgM RF titers (κ: 0.46, $p<0.001$). Such association was reported by Greiner et al (6), whereas Agrawal et al (18) reported a significant correlation between IgA RF and IgM RF levels and also between IgA RF and anti-CCP levels. But, they didn't find any correlation between anti-CCP and IgM RF levels.

Our study showed that IgM RF positivity was related to accelerated ESR, which is a major serological marker of disease activity. Joint tenderness and swelling were found to be principally associated with anti-CCP and IgM RF. However, none of the tested antibodies had any significant correlation with disease activity score. Controversy exists regarding the association between autoantibodies positivity and disease activity. Thus, according to some authors (19) raised IgA RF level is a reliable marker of disease activity. In a more recent study anti-CCP were better serum markers of disease activity than IgM and IgA RF (4). The study by Bas et al (7), in which IgM RF, IgA RF and anti-CCP were investigated, showed that only patients with RF had significantly higher DAS. Similarly, Alexiou et al (11) showed that serum IgM RF levels had a correlation with DAS28 score but they didn't find any correlation for anti-CCP. Likewise, Greiner et al (6) didn't find any significant relationship between anti-CCP, IgM or IgA RF titers and diverse serological markers of disease activity.

In our study, subcutaneous nodules were present in only 13 subjects. There was no correlation between this extrarticular manifestation and positivity of any of tested antibodies. Such association was reported by several studies. In deed, Turesson et al (20) reported a strong association between IgM RF and severe extra articular mafenistations in RA, they also reported a weaker association for anti-CCP. Bas et al, found that IgM RF, but not anti-CCP was significantly more frequent among patients who had subcutaneous nodules (21).

The RA-characteristic joint destruction occurs in the majority of patients within the first two years of the disease. Numerous studies have found links between anti-CCP antibodies and joint damage during this disease evolution stage (13, 22, 23). Earlier studies have reported an association between IgA RF and RA with increased joint damage and poor prognosis. (19, 24, 25). In the study of Shankar et al (26) patients positive for both IgM RF and anti-CCP were found to have a higher prevalence of erosions as compared to patients positive for only one antibody or negative for both. These data are in agreement with the findings of Mewar et al (27), which indicate the independent associations of RF and anti-CCP with radiological severity of RA. Other authors (18) revealed that association with severe and erosive disease was stronger for AKA than for IgM RF and anti-CCP. Our results suggest that anti-CCP are the most important serological marker reflecting joint damage.

In conclusion, the presence of antibodies against CCP is associated with RA severity and could lead to a change in management of patients previously classified as having mild disease. Nevertheless, in long duration disease anti-CCP can't replace IgM as a screening marker in our everyday practice. In deed, anti-CCP have much higher costs compared with rheumatoid factor testing and both have similar diagnostic value. Thus, we estimate that IgM RF is a good first line test for RA, and that anti-CCP are useful in ambiguous cases or in IgM RF- negative patients.

References

1. Lee DM, Weinblatt ME. Rheumatoid arthritis. Lancet 2001; 358: 903-11.
2. NG K, Austin P, Ameratunga R, McQueen F. Role of anticyclic citrullinated peptide 2 assay in long-standing rheumatoid arthritis. APLAR Journal of Rheumatology 2006; 9: 211-5.
3. Arnett FC, Edworthy SM, Bloch DA et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31: 315-24.
4. Vallbracht I, Rieber J, Oppermann M, Förger F, Siebert U, Helmke K. Diagnostic and clinical value of anti-cyclic citrullinated peptide antibodies compared with rheumatoid factor isotypes in rheumatoid arthritis. Ann Rheum Dis 2004; 63: 1079-84.
5. Meyer O, Fabregas D, Cyna L, Ryckewaert A. Anti-keratin antibodies. A marker of progressive rheumatoid arthritis. Rev Rhum Mal Osteoartic 1986; 53: 601-5.
6. Greiner A, Plischke H, Kellner H, Gruber R. Association of anti-cyclic citrullinated peptide antibodies, anti-citrullin antibodies, and IgM and IgA rheumatoid factors with serological parameters of disease activity in rheumatoid arthritis. Ann N Y Acad Sci 2005; 1050: 295-303.
7. Bas S, Genevay S, Meyer O, Gabay C. Anti-cyclic citrullinated peptide antibodies, IgM and IgA rheumatoid factors in the diagnosis and prognosis of rheumatoid arthritis. Rheumatology 2003; 42: 677-80.
8. Prevoo ML, Van't Hof MA, Kuper HH, Van Leeuwen MA, Van de Putte LB, Van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995; 38: 44–8.
9. Zendman AJ, Van Venrooij WJ, Pruijn GJ. Use and significance of anti-CCP autoantibodies in rheumatoid arthritis. Rheumatology 2006; 45: 20-5. Review.
10. Niewold TB, Harrison MJ, Paget SA. Anti-CCP antibody testing as a diagnostic and prognostic tool in rheumatoid arthritis.QJM 2007; 100: 193-201.
11. Alexiou I, Germenis A, Ziogas A, Theodoridou K, Sakkas LI. Diagnostic value of anti-cyclic citrullinated peptide antibodies in Greek patients with rheumatoid arthritis. BMC Musculoskelet Disord 2007; 8:37.
12. Lee DM, Schur PH. Clinical utility of the anti-CCP assay in patients with rheumatic diseases. Ann Rheum Dis 2003; 62: 870-4.
13. Kroot EJ, De Jong BA, Van Leeuwen MA et al. The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. Arthritis Rheum 2000; 43: 1831-5.
14. Solanki K, Spellerberg M, Chapman P, Moller P, O'Donnell J. Anti-cyclic citrullinated antibodies: complementary to IgM rheumatoid factor in the early diagnosis of rheumatoid arthritis. N Z Med J 2004; 117: U1097.
15. Ordeig J, Guardia J. Diagnostic value of anti-keratin antibodies in rheumatoid arthritis. J Rheumatol 1984; 11: 602-4.
16. Meyer O, Combe B, Elias A et al. Autoantibodies predicting the outcome of rheumatoid arthritis: evaluation in two subsets of patients according to severity of radiographic damage. Ann Rheum Dis 1997; 56: 682-5.
17. Vasiliauskiene L, Wiik A, Hoier-Madsen M. Prevalence and clinical significance of anti-keratin antibodies and other serological markers in Lithuanian patients with rheumatoid arthritis. Ann Rheum Dis 2001; 60: 459-66.
18. Agrawal S, Misra R, Aggarwal A. Autoantibodies in rheumatoid arthritis: association with severity of disease in established RA. Clin Rheumatol 2007; 26: 201–4.
19. Päi S, Päi L, Birkenfeldt R. Correlation of serum IgA rheumatoid factor levels with disease severity in rheumatoid arthritis. Scand J Rheumatol 1998; 27: 252-6.
20. Turesson C, Jacobsson LT, Sturfelt G, Matteson EL, Mathsson L, Rönnelid J. Rheumatoid factor and antibodies to cyclic citrullinated peptides are associated with severe extra-articular manifestations in rheumatoid arthritis. Ann Rheum Dis 2007; 66: 59-64.
21. Bas S, Perneger TV, Seitz M, Tiercy JM, Roux-Lombard P, Guerne PA. Diagnostic tests for rheumatoid arthritis: comparison of anti-cyclic citrullinated peptide antibodies, anti-keratin antibodies and IgM rheumatoid factors. Rheumotology 2002; 41: 809-814.
22. Forslind K, Ahlmén M, Eberhardt K, Hafström I, Svensson B. Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides (anti-CCP). Ann Rheum Dis 2004; 63: 1090–5.
23. Ronnelid J, Wick MC, Lampa J et al. Longitudinal analysis of citrullinated protein/peptide antibodies (anti-CP) during 5 year follow up in early rheumatoid arthritis: anti-CP status predicts worse disease activity and greater radiological progression. Ann Rheum Dis 2005; 64: 1744–9.
24. Teitsson I, Withrington RH, Seifert MH, Valdimarsson H. Prospective study of early rheumatoid arthritis. I. Prognostic value of IgA rheumatoid factor. Ann Rheum Dis 1984; 43: 673–678.
25. Tarkowski A, Nilsson L A. Isotype specific measurement of rheumatoid factor with reference to clinical features of rheumatoidarthritis. J Clin Lab Immunol 1983; 12: 129-35.
26. Shankar S, Grover R, Handa R. Role of anti cyclic citrullinated peptide antibodies in erosive disease in patients with rheumatoid arthritis. Indian J Med Res 2006; 124: 689-96.
27. Mewar D, Coote A, Moore DJ et al. Independent associations of anti-cyclic citrullinated peptide antibodies and rheumatoid factor with radiographic severity of rheumatoid arthritis. Arthritis Res Ther 2006; 8: R128.