Synovial Fluid Levels Of Anti-Cyclic Citrullinated Peptide Antibodies And Iga Rheumatoid Factor In Rheumatoid Arthritis And Osteoarthritis

By

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ABSTRACT:

Objectives: To assess the levels of anticyclic citrullinated peptide antibodies (anti-CCP) and IgA rheumatoid factor (IgA-RF) in synovial fluids of patients with rheumatoid arthritis and osteoarthritis (OA).

Methods: Knee effusions of 19 patients with RA (16 women, 3 men), mean age 39.95±12.8 years, 24 patients with OA (19 women, 5 males) with a mean age of 43.4±11.7 years, were aspirated, centrifuged and stored at -20^{o}C. Sera of patients with RA and OA were similary stored, IgG anti-CCP and IgA RF were detected by enzyme linked immunosorbant assay. Different clinical and laboratory characteristics were assessed.

Results: Mean levels of synovial fluid anti-CCP and IgA-RF were significantly, increased in RA joint effusions compared with that in OA. (anti-CCP was 87.84±117.75 and 9.75±18.08 units), respectively ($P < 0.001$). IgA RF was (61.11±87.8 and 8.08±9.64) respectively, ($P< 0.001$). A significant correlation was found between synovial fluid anti-CCP and serum anti-CCP and IgA-RF. Significant correlation was also found between synovial fluid IgA-RF and erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) but not with synovial anti-CCP.

Conclusion: Anti-CCP and IgA-RF were significantly increased in synovial fluid of patients with RA in comparison with patients having OA.

Key words:

Rheumatoid arthritis (RA), Osteoarthritis (OA), Synovial fluid (SF) anti-cyclic citrullinated anti-bodies (anti-CCP), IgA rheumatoid factor (IgA-RF).

INTRODUCTION:

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of the joints, eventually resulting in erosive changes and joint deformities. The diagnosis of RA is primarily based on clinical manifestations of the disease, supported in many cases by serologic findings. Rheumatoid factor is present in up to 70% of patients but is also detected in a variety of auto-immune disorders, other non rheumatic conditions and in healthy individuals ***(Smolen, 1996).***

For years serologic support in the diagnosis of RA has been limited to the presence of rheumatoid factors, although not very specific for RA ***(Vallbracht and Helmike, 2005).***

During the last years a variety of circulating non-RF antibodies have been discovered and reported to be of potential diagnostic value ***(Bas et al., 2003)***. Although the precise mechanisms responsible for the formation of these antibodies have not been well defined, their presence must reflect the interaction between T and B-cells believed to be relevant to the pathogenesis of RA ***(Mewan and Wilson, 2006).***

Among the antibodies described in recent years the most promising candidates are the autoantibodies to antigens containing one or more than one citrulline residues (cyclic citrulline peptides, CCP) the anti-CCP antibodies. They have been shown to play an important role in the

diagnosis, prognosis and therapeutic approach to patient with RA ***(Bizzaro, 2007).***

According to the specific test used, antibodies directed towards citrullinated proteins have been described in 60-70% of patients with RA with a specificity of 85-95% ***(Vossenaar and Van, 2004 and Nishimura et al., 2007).***

Citrullinated proteins seem to originate in the synovium ***(Vossenaar et al., 2003),*** and anti-cyclic citrullinated proteins (anti-CCP) appear to be produced in the inflamed synovium by local plasma cells ***(Mason et al., 2000).***

Furthermore, anti-CCP producing B-cells have been detected in the synovial fluid of anti-CCP positive patients with RA ***(Reparon et al., 2001).***

Although citrullinated proteins are present in the synovium of mice with collagen induced arthritis, the induction of autoantibodies directed to these proteins is a more specific phenomenon detectable only in human patients with RA ***(Vossenaar, et al., 2003).***

It has been shown that the presence of citrullinated peptides is not specific to the rheumatoid synovium and may be locally detected in other inflammatory arthropathies ***(Vander Cruyessen et al., 2005 and Vossenaar, 2004).***

Therefore, the presence of CCPs in the synovium of different arthropathies does not preclude specific local humoral response of the inflamed synovium to CCP, which may vary in different arthritides or be correlated with the degree of inflammation ***(Caspi et al., 2006).***

The aim of this study was to measure the levels of anti-CCP and IgA-RF in joint effusions of patients with RA, OA and to evaluate the specificity and possible diagnostic value of anti-CCP and IgA-RF as well as their correlation with different clinical and laboratory data.

PATIENTS AND METHODS

Nineteen patients with RA and 24 patients with OA were included in this study. All the patients had knee aspiration due to acute synovitis. The synovial fluid samples were centrifuged and the supernatant was frozen at -20^{o}.

Anti-CCP enzyme linked immunosorbent assay: IgG anti-CCP of the synovial fluid and serum was performed using a second generation commercial kit (Quanta Lite; Inova Diagnostics, san Diego, CA). Serial dilution of the synovial fluid samples were diluted 1:10, 1:100 and 1:1,000). Synovial fluid samples were diluted 1:10 and serum samples 1:100. Samples were considered weekly positive if the antibody titer was between 20 and 39 IU, moderately positive between 40 and 59 IU; and strongly positive $\geq$ 60 IU).

IgA rheumatoid factor: IgA-RF of the synovial fluid and serum was performed using a commercial kit (Quanta Lite Inova Diagnostics), according to the manufacturer's instructions. Synovial fluid samples were diluted 1:10 and serum samples 1:100. Samples were considered positive if the antibody titer was $\geq$ 6 IU. Various clinical and laboratory data of the patients were collected.

STATISTICAL ANALYSIS:

The data was coded and entered using the statistical package SBSS version 12. The data was summarized using mean and standard deviation (SD) for the quantitative variables and percentage for qualitative variables. Comparison between groups were done using chi-square test for qualitative variable and non parametric Mann-Whitney test for quantitative data. Correlation was done to show the relation between quantitative variables. P-value < 0.05 was considered as statistically

significant. ROC (receiver operating curve) was done to show the validity of different variables (IgA-RF and anti-CCP) to detect patients with RA.

RESULTS:

The demographic characteristics of the patients are summarized in **Table (1):**

Characteristic	**Rheumatoid arthritis**	**Osteoarthritis**
Female / male	16 / 3	19 / 5
Age mean ±SD year	39.95 ± 12.76	43.46 ± 11.74
Disease duration mean ±SD year	8.26 ± 4.62	5.2 ± 8

Most of RA patients were women as well as patients with OA. Patients with OA were older than RA patients with mean age ±SD 43.46±11.74, 39.95±12.76 years respectively. As regards disease duration, it was 8.26±4.6 years in RA patients and 5.2±8 years of OA patients.

Seventy three percent (73.7%) of patients with RA were seropositive for IgM-RF whereas OA patients were seronegative.

Table (2): Different clinical and laboratory parameters for patients with RA

Characteristics	**Mean ±SD**
Morning stiffness (MS)	0.96 ± 0.86
ESR	77.37 ± 30.69
DAS 28	4.57 ± 0.94
Platelets	423.68 ± 145.4
CRP	18.35 ± 24.29

Levels of anti-CCP and IgA-RF in the serum and synovial fluids of the patients:

The mean level ±SD of synovial fluid anti-CCP and IgA-RF were significantly increased in RA joint effusion in comparison with OA. Anti-CCP level was (87.84±117.43, 9.75±18 units) respectively, P< 0.001 **(Fig. 1).**

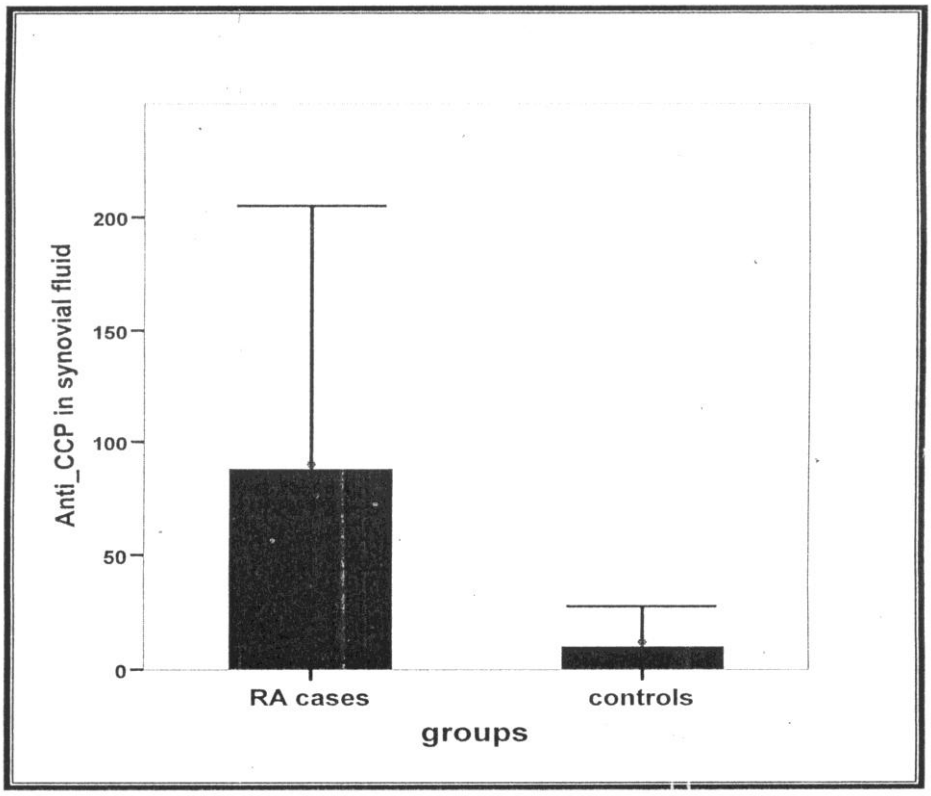

Fig. (1): Comparison between synovial anti-CCP between patients with RA and patients with OA

The mean level ±SD of IgA-RF was 61.11±87.88 units in RA patients Which was significantly higher than IgA-RF level in synovial fluid of OA patients (8.08±9.64 units) P < 0.001 **(Fig. 2)**.

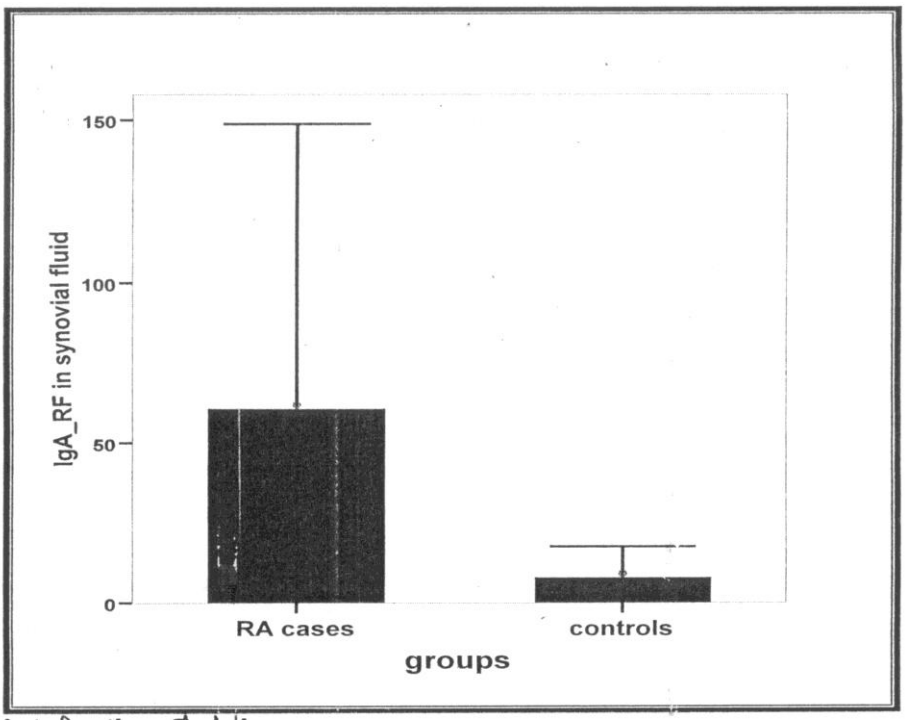

Fig. (2): Comparison of synovial IgA-RF between patients with RA and OA

The serum values of both tests were also significantly higher in RA patients than in the OA patients (P-value < 0.05) **(Table 3)**.

Table (3): Values of anti-CCP and IgA-RF in synovial fluid and serum of patients with rheumatoid arthritis and osteoarthritis

Characteristic	**Rheumatoid arthritis**	**Osteoarthritis**
Synovial fluid anti-CCP	87.87 ± 112.48	9.45 ± 18.0
Serum anti-CCP	104.58 ± 109.69	9.92 ± 7.5
Synovial fluid IgA-RF	61.11 ± 87.88	8.08 ± 9.64
Serum IgA-RF	58.95 ± 70.34	14.04 ± 10.20

A positive correlation was found between anti-CCP level in serum and synovial fluid in RA patients (P < 0.001) **(Fig. 3)**.

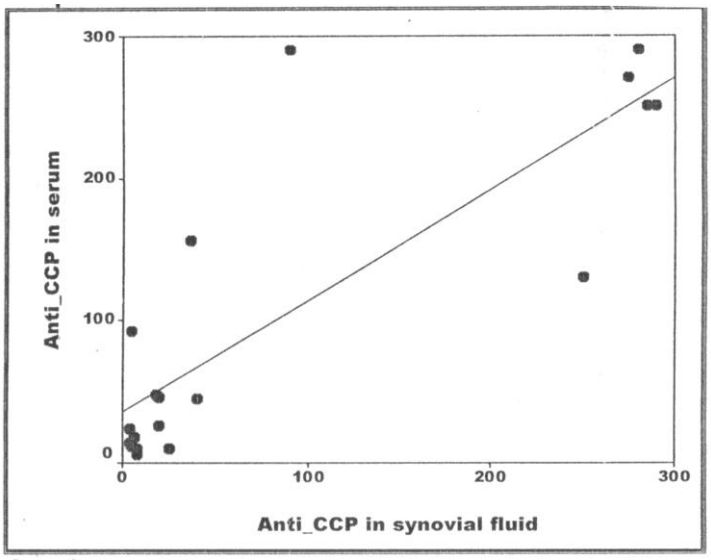

Fig. (3): Correlation between anti-CCP in serum and synovial fluid

A positive correlation was found between serum and synovial IgA-RF P<0.001 **(Fig. 4).**

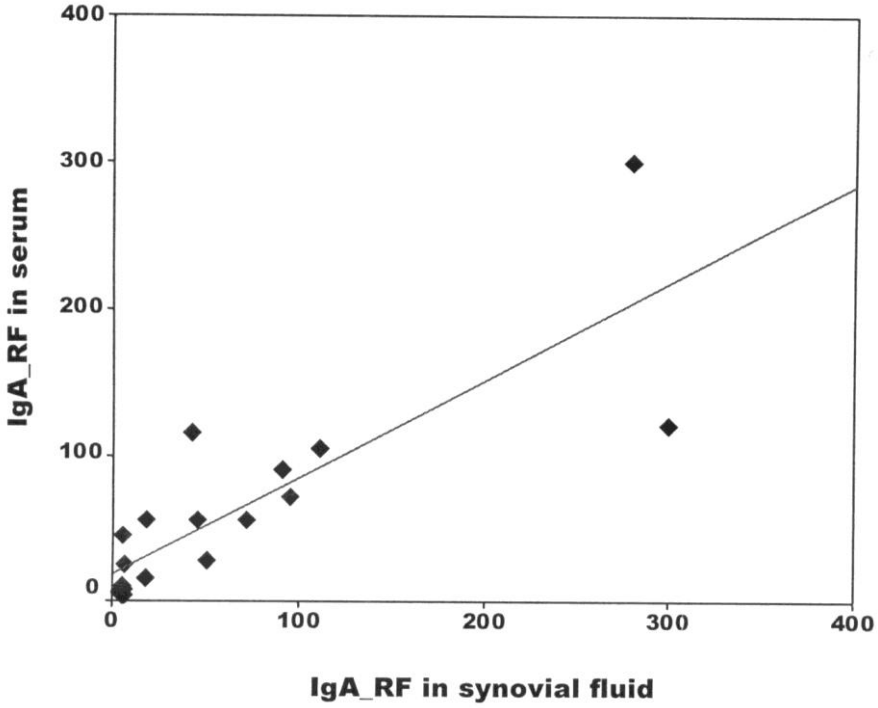

Fig. (4): Correlation between serum and synovial IgA-RF

Different correlation between serum and synovial anti-CCP and IgA-RF are illustrated in **(Table 4)**.

Serum anti-CCP was correlated with serum and synovial IgA-RF, ($P < 0.05$) while synovial anti-CCP was correlated with serum IgA-RF.

Serum IgA-RF was correlated with serum and synovial anti-CCP ($P<0.05$), while synovial IgA-RF was correlated with serum anti-CCP.

Table (4): Correlation between serum and synovial anti-CCP in IGA-RF

		Synovial CCP	**Serum CCP**	**Synovial IgA**	**Serum IgA**
Synovial anti-CCP	Pearson correlation Sig. (2-tailed)	1 0	0.837** 0.000	0.288 0.232	0.458* 0.049
Serum anti-CCP	Pearson correlation Sig. (2-tailed)	0.837** 0.00	1 0	0.569* 0.011	0.481* 0.037
Synovial IgA-RF	Pearson correlation Sig. (2-tailed)	0.288 0.232	0.569* 0.011	1 0	0.832 0.000
Serum IgA-RF	Pearson correlation Sig. (2-tailed)	0.458* 0.049	0.481* 0.037	0.832 0.000	1 0

ESR level was found to be correlated with synovial IgA-RF ($P < 0.05$) but not with synovial anti-CCP. The same with CRP level which was found to be correlated with synovial IgA-RF ($P < 0.05$) but not with synovial anti-CCP.

IgM-RF was found be correlated with serum and synovial IgA-RF ($P < 0.001$).

Sensitivity and specificity of serum and synovial fluid levels of anti-CCP and IgA-RF:

ROC curve was done and areas under curve were calculated to show the ability of serum and synovial IgA-RF and anti-CCP to detect

RA patients. Area under the curve was found to be (0.89, 0.81) for serum and synovial anti-CCP and (0.72, 0.81) for serum and synovial IgA-RF and CRP (0.84) **(Fig. 5)**.

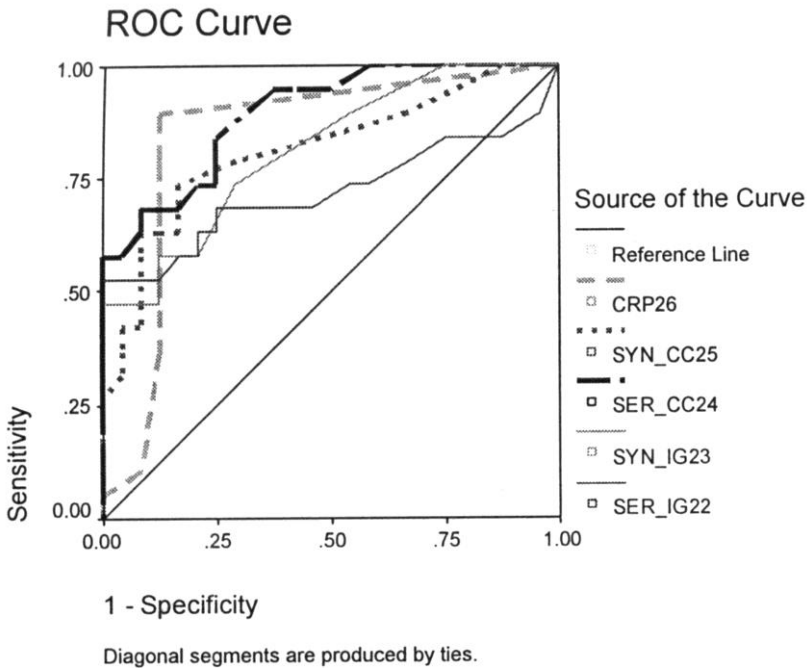

Fig. (5): ROC curve to show the validity of different variables to detect RA patients

<u>The sensitivity and specificity of serum and synovival fluid levels of anti-CCP and IgA-RF:</u>

Serum anti-CCP levels $\geq$ 40 IU (moderately and strongly positive) according to the recommendations of the manufacturer (Inova diagnostics) had a sensitivity of 52% with a specificity of 100% for the diagnosis of RA. For the synovial anti-CCP, sensitivity was 31% and specificity was 96% for the diagnosis of RA.

Combining both serum and synovial anti-CCP showed a sensitivity of 57.9% and a specificity of 95.8%. For IgA-RF the sensitivity and specificity of serum IgA-RF (using serum levels $\geq$ 6 IU) was 84% and 25% respectively while for synovial IgA-RF, it was 57% and 80%.

Combining both serum and synovial IgA-RF showed a sensitivity of 89.5% and a specificity of 12.5%.

Combining both synovial anti-CCP and IgA-RF gave a sensitivity of 78.9% and a specificity of 70.8%.

DISCUSSION

Antibodies directed towards citrullinated proteins are highly specific for RA, but their sensitivity is around 65-75% ***(Schellekens et al., 2000 and Vossenaar et al., 2004).***

Although it was previously thought that the presence of tissue citrullinated proteins in the synovium is characteristic of RA ***(Baeten et al., 2001).*** It has been shown that these proteins are also found in the synovium of other arthritides such OA, reactive arthritis ***(Vossenaar et al., 2004)*** and psoriatic arthritis ***(Vandercruyessen et al., 2005).***

In our study, we have shown that levels of anti-CCP and IgA-RF are significantly increased in the serum and synovial fluid of patients with RA in comparison with synovial fluid of OA patients.

The significant finding of increased anti-CCP levels in the synovial fluid of RA patients suggests that it's the humoral immunologic response to these proteins that characterizes RA rather than the presence of the triggering antigen in the synovium ***(Caspi et al., 2006).***

Spadaro et al., (2006) mentioned that their study gave evidence for a preferential production of anti-CCP at RA joint level confirming the pathogenic role of these autoantibodies.

In our study, we had found a significant correlation between serum and synovial anti-CCP in RA patients. The capacity to develop these antibodies to CCP seems to reflect the interaction between T and B cells believed to be relevant to the pathogenesis of RA ***(Mewan and Wilson, 2006).***

De Rycke et al., (2006) also emphasized on the pathophysiologic relevance of anti-CCP.

RF constitutes one of the diagnostic criteria of RA proposed by the American College of rheumatology (ACR) ***(Arnett et al., 1988).*** However, RF positivity shows low diagnostic specificity ***(Smolen, 1996).***

Among the various RFs, IgA-RF has a similar sensitivity to IgM-RF ***(Difranco et al., 1999)*** but has a higher correlation with extra-articular manifestation of RA, including sicca syndrome ***(Johnson et al., 1995)*** and with erosive disease ***(Jorgensen et al., 1996).***

In our study, IgM-RF was found to be correlated with serum and synovial IgA-RF ($P<0.001$).

Also in our study, we had found better specificity for synovial IgA-RF (80%) compared to serum IgA-RF (25%). However, serum IgA-RF has a better sensitivity than synovial IgA-RF, (84%) and (57%) respectively.

Through this study, anti-CCP was found to be correlated with IgA-RF and this is the same found by ***Kastbom et al., in (2004).***

ESR and CRP levels were found to be correlated with the synovial IgA-RF but not with synovial anti-CCP ($P<0.05$).

Shovman et al. (2005) didn't find any correlation between anti-CCP antibody titers and inflammatory markers such as ESR or CRP.

We didn't find any correlation between DAS28, extra-articular manifestations of RA patients and anti-CCP level.

Also we didn't find any correlation between erosive arthritis (by x-ray imaging using sharp score) and anti-CCP level.

Spadaro et al., in (2006) didn't find any correlation between anti-CCP level and different clinical data. ***Vallbact and Helmke (2005)*** showed that the anti-CCP to be a good prognostic marker as it can predict the erosive or non-erosive progression of the disease.

Van der Helm-Van Mil et al., (2005) showed that RA patients with anti-CCP antibodies had more severe radiological desturction at follow-up.

We had calculated the sensitivity and specificity of both serum and synovial anti-CCP and IgA-RF (using the cut off values recommended by the manufacturer) (Inova diagnostics) for anti-CCP, it showed a highly specific marker to diagnose RA patients although it's less sensitive. Combining anti-CCP test with IgA-RF increased the sensitivity of the test to diagnose RA and this agrees with ***Caspi et al., (2006)*** who also stated that the sensitivity of anti-CCP is increased when combined with IgA-RF in the diagnosis of RA.

Synovial IgA-RF had a higher specificity (80%) compared with serum IgA-RF (25%) and it was less sensitive in diagnosing patients with RA than serum IgA-RF.

Data on the presence and significance of RFs in the snovial fluid is scarse. IgM-RF and IgG-RF have been detected in the synovial fluid of patients with RA ***(Lettesjo et al., 1998)*** but there are no data concerning IgA-RF although it has been shown that synovial cells have the capacity to produce IgA-RF in vitro ***(Caspi et al., 2006).***

Similarly data on anti-CCP in the synovial fluid is scarce ***(Caspi et al., 2006 and Spadaro et al., 2006)*** and concern mainly the synovial tissue ***(Vossenaar et al., 2004).***

CONCLUSION

In our present study, we have demonstrated the importance of anti-CCP in the synovial fluid of patients with RA as well as IgA-RF, in the diagnosis of RA, although it is limited by the relatively small number of patients.

It's significantly increased in comparison with that of patients with OA. Synovial fluid anti-CCP clearly correlates with serum anti-CCP. Serum anti-CCP was found to be correlated with serum and synovial IgA-RF. Synovial anti-CCP was correlated with serum IgA-RF. We recommend to study synovial anti-CCP in other inflammatory arthritides for further documentation of its importance.

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