

IS ANTI CYCLIC CITRULLINATED PEPTIDE A USEFUL LABORATORY TEST FOR THE DIAGNOSIS OF RHEUMATOID ARTHRITIS?

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Summary

■ **Aim of the study:** To evaluate Anti Cyclic citrullinated peptide (anti-CCP) and Rheumatoid Factor (RF) sensitivity and specificity in patients with Rheumatoid Arthritis (RA).

■ **Materials and methods:** Anti-CCP and RAtest for the assessment of RF have been tried out in 35 patients with RA and in a group of 42 patients whose hands were affected by arthralgic/arthritis-like symptoms.

■ **Results:** Sensitivity of anti-CCP was 71,4% with 95,2% of specificity. RAtest sensitivity was 91,4% with specificity of 31%. Anti-CCP and RAtest both positive showed an overall 71.4% sensitivity and 95.2% specificity; sensitivity was 91.4% and specificity was 31.0%. if at least RAtest or anti-CCP resulted positive, The positive predictive value of the two test performed together was 97.0%, higher than that of anti-CCP (71.4%) and RAtest (88.5%) alone. The negative predictive value of the two test performed together was 30.9% similar to that of the RAtest performed alone (30.9%). All patients affected by HCV related chronic liver disease were negative for anti-CCP test; 12/14 of them had RAtest positive.

■ **Conclusions:** Anti-CCP antibody has a diagnostic specificity higher than RF. Anti-CCP and RA tests together prove to have the same specificity as anti-CCP alone and a lower sensitivity. Sensitivity is better when at least either RAtest or anti-CCP results positive. The best positive predictive value is obtained performing both anti-CCP and RAtest.

Anti-CCP is a useful laboratory marker to confirm the diagnosis of rheumatoid arthritis; it seems to be very important to differentiate patients with RA from those with HCV related arthritis.

Key-words: Anti Cyclic Citrullinated Peptide - Rheumatoid Factor - Rheumatoid Arthritis - HCV related chronic liver disease.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease affecting about 1% of the adult population; mainly, it involves joints leading to anatomical damage within the first two years from the onset, with consequent different degrees of disability (1-2). Making a correct diagnosis in the early stages of the disease allows us to apply powerful therapies and delay the onset of articular damage, with patient's prognosis improvement (3).

The diagnosis of RA is mainly based on clinical criteria, whereas laboratory tests contribute rather slightly.

The most frequently used laboratory test for the diagnosis of RA is the determination of serum rheumatoid factor (RF), which is believed to play a very good prognostic role too (4-5). RF is a non specific test because it can be detected in many other diseases, among which HCV related arthritis (6), generally through the RAtest (7).

The detection of antibodies directed towards cyclic citrullinated peptide (CCP) is a test that has been recently introduced into clinical practice, and that has been given high diagnostic significance. Anti-CCP recognizes a modified aminoacid considered the highly purified synthetic form of the antigen identified, in immunofluorescence, by antifilaggrin antibodies (AFA) (8). It is possible that citrullination, carried out by an enzyme that appears to be hormonally controlled, open new possibilities for studying the aethiology of RA (9).

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A number of reports credit the anti-CCP with a diagnostic specificity rate (up to 98%) higher than the one of RF, along with a fairly low sensitivity (10). Moreover, anti-CCP seems to play a very useful role as a prognostic factor, being able to identify the most disabling forms of RA (11-13). The aim of our study is to evaluate the sensitivity of anti-CCP and of RA test in patients with RA and other diseases, among which HCV related arthritis.

MATERIALS AND METHODS

PATIENTS

Serum samples were collected from 35 consecutively patients with RA (Group A, 27 F, 8 M, mean age 70 years) and 42 non-RA control patients sent, in the same period, to our team for arthralgias/arthritis particularly affecting their hands (group B 33F, 9M, mean age 57 years). In the control group there were included: HCV related chronic liver disease (n=14), hand osteoarthritis and post-infective

polyarthritis (n=5), Hashimoto's thyroiditis and polymyalgia rheumatica (n=4), primary Sjogren's syndrome, systemic lupus erythematosus, seronegative spondyloarthritis and calcium pyrophosphate deposition disease (n=2), systemic sclerosis and paraneoplastic polyarthritis (n=1).

The diagnosis of RA was based on 1987 revised ARA criteria (14). The characteristics of the patients of both groups are reported in table 1.

All the patients (groups A+B) were tested for HCV antibodies (third generation ELISA) and HbsAg. If turned out positive, liver function test and viral HCV-RNA (nested PCR) were checked. Liver biopsy was proven in patients with persistent elevated S-ALT and HCV-RNA positive patients. In patients positive to HBsAg, Anti-HBe, antigen and antibodies were researched.

ANTI-CCP ANTIBODIES ASSAY

The IgG anti-CCP evaluation was performed on sera samples that have been kept frozen at -20°C before

Diagnosis	Patients	Anti-CCP +	RA test +	
RA	35 (27F; 8M)	25	32	Sensitivity anti-CCP 71.4%
HCV related chronic liver disease	14	0	12	
Hand osteoarthritis	5	0	3	
Postinfective polyarthritis	2 Parvovirus B19 1 Mycoplasma pneumoniae 2 aspecific	0 0 0	0 1 1	
Hashimoto's thyroiditis	4	2	3	Specificity 95.2%
Polymyalgia 3 PMR rheumatica	1 Horton arthritis	0	3	
Crystal pyrophosphate deposition disease	2	0	1	
Seronegative spondyloarthritis	2	0	0	
SLE	2	0	2	
Primary Sjogren Syndrome	2	0	2	
Scleroderma Paraneoplastic	1	0	1	
Polyarthritis	1 (Lung cancer)	0	0	

Table 1.

testing. Anti-CCP antibodies were determined by a second-generation of sandwich enzyme linked immunosorbent assay (anti-CCP ELISA, AXIS SHIELD, Dundee, UK) according to the manufacturer's instructions. The obtained results were expressed as positive or negative, according to the standard values shown in the kit (<5 U/ml).

IGM RF ANTIBODIES ASSAY

RF was noticed by using RAtest, (N latex RF, DADE BEHRING, Marburg, Germany) the nephelometric determination of the agglutination of latex particles sensitized with human and animal gammaglobulines and reacting with the Rheumatoid Factors present in the serum (n.v. < or =20 U/ml).

Informed consent was obtained by all patients for the determination of the tests.

RESULTS

A female patient, belonging to the A group, was found positive to HCV Ab test twice; SGOT and SGPT were normal and viral RNA check was negative.

A male patient from the B group was found positive to HBs Ag; HBeAg was negative and HBeAb positive; S-ALT and S-AST were normal and no more evaluations were needed.

About the group B fourteen patients had HCV antibodies; in eleven of them, who had persistently elevated S-ALT and resulted positive for HCV-RNA, liver biopsy was performed that revealed chronic hepatitis (5F, 6M mean age 41.3 years).

Anti-CCP turned out positive in 25/35 patients of the A group (sensitivity 71.4%, CI 56.4%-86.4%) and negative in 40 patients of the B group (specificity 95.2%, CI 88.7%-100%). RAtest sensitivity is 91.4% (CI 82.1%-100%) with 31% (CI 17%-45%) specificity (table. 2).

Anti-CCP along with RA has 71.4% sensitivity (CI 56.4%-88.7%) and 95.2% specificity (CI 88.7%-100%); the presence of anti-CCP or RAtest has 91.4% sensitivity (CI 82.1%-100%) and 31.0% specificity (CI 17.0%-45.0%). The positive predictive value of anti-CCP and RAtest performed separately was 71.4% and 88.5% respectively; their negative predictive values were 95.2% and 30.9% respectively. The two tests performed together showed a positive predictive value of 97.0% and a negative predictive value of 39%. 12/14

of patients affected by HCV related arthritis (85.7%) were RAtest positive, but all of them were anti-CCP negative. Two patients (50%) with Hashimoto's thyroiditis and positive to RAtest resulted also positive to anti-CCP.

Test	Sensitivity	Specificity
Anti-CCP	71.4% (CI 56.4%-86.4%)	95.2% (CI 88.7%-100%)
RAtest	91.4% (CI 82.1%-100%)	31% (CI 17.0%-45.0%)
RAtest and anti-CCP	71.4% (CI 56.4%-86.4%)	95.2% (CI 88.7%-100%)
RAtest or anti-CCP	91.4% (CI 82.1%-100%)	31% (CI 17.0%-45.0%)

Table 2 : Anti-CCP and RAtest sensitivity and specificity.

DISCUSSION

Together with well-defined clinical and radiological criteria, RF is the autoantibody currently used for the diagnosis of RA (13), mainly via the RAtest. It is not clear yet, whether exist different RF forms, few of which pathogenic. It is furthermore difficult to characterize the RFs *in vivo*, because of their polyclonality, specificity and avidity, which are the same intrinsic features giving them their pathogenic activity (15).

Cyclic citrullinated peptide, obtained through a post-transcriptional enzymatic substitution of arginin residuals with citrullin, is the substrate of a second generation ELISA test for the detection of anti-CCP antibodies; this test seems to offer good perspectives, both for early diagnosis (16-17) and, according to some authors, in predicting the most aggressive forms of the disease (18-19).

Anti-CCP could have also an effective role in the pathogenesis of RA, as suggested by the fact the some citrullinated proteins have been found in the articular space of diseased joints (21).

Data from the present study confirm the high specificity of anti-CCP, together with a fair sensitivity. The determination of both anti-CCP and RAtest makes an improvement of sensitivity, which is higher when at least one of the two tests results positive. The good specificity of anti-CCP resulted also in a high positive predictive value (71.4%); this was lower than that of RAtest (88.5%) and of the association of both tests (97.0%): this result minimizes the number of false positive patients.

Polyarthritis, in association with positive IgM RF, is frequently described in patients with chronic HCV infection thus making the differential diagnosis between HCV related arthritis and true RA difficult in some cases (21). In the experience of Fadda P. (22) the

arthritis of patients with chronic HCV related hepatitis is symmetrical (86%), polyarticular (85%) and, in some cases, erosive; it is also characterized by the presence of RF in 55% of the patients. Toubi et al found that IgM RF was present in 42% of patients with chronic HCV infection. (23). The detection of serum RF cannot be used as a laboratory marker for the differential diagnosis between HCV related arthritis and RA. In an experience of Kessel A. the detection of antikeratin antibodies (AKA) was considered useful for this purpose (24). AKA are detected by indirect immunofluorescence technique on rat oesophagus as antigen and have been found positive in 64% of RF-positive and in 28% of RF-negative patients with RA; moreover, they could correlate with the severity of the disease (25). In our study it is the first time that anti-CCP is used as a useful instrument to distinguish RA from HCV related arthritis.

Anti-CCP have higher specificity than AKA, are directed towards the same epitopes recognized by AKA and are detected in ELISA technique, that is more reliable than immunofluorescence (26). We found actually that all the 12 RF-positive patients affected by HCV related arthritis resulted negative to anti-CCP.

According to the results of our study, anti-CCP is confirmed to be the laboratory test able to discriminate patients with RA better than RAtest; the determination of both anti-CCP and RAtest makes it possible to further improve the sensitivity and the positive predictive value of laboratory tests; more studies will be necessary to confirm the available data at present, above all in patients with polyarthritis of recent onset, for whom an early diagnosis makes it possible to apply the most suitable drug therapy.

It will be also necessary to ascertain which prognostic role anti-CCP could play in the discrimination of the most aggressive forms of RA from the very start, both from radiographic and disability point of view.

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