

Anti-CCP antibodies have more diagnostic impact than rheumatoid factor (RF) in a population tested for RF: Impact of including anti-CCP to the diagnostic criteria for early Rheumatoid Arthritis

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ABSTRACT

Purpose: To determine the clinical utility of anti-cyclic citrullinated peptide (CCP) antibody in a population where RF was ordered. Both tests would likely be ordered together if anti-CCP antibody were included in the criteria to diagnose early rheumatoid arthritis (RA).

Materials and Methods: Informed consent to test for anti-CCP was obtained from 1025 consecutive patients for whom rheumatoid factor (RF) was ordered at the University Central Hospital Laboratory. The RF requests originated from primary, secondary and tertiary centers. A diagnosis was obtained from the physicians without telling them the anti-CCP result. After exclusion criteria were applied, 768 patients composed the final study population.

Results: In this group, 132 people (17%) were diagnosed with RA. The sensitivity for RA of anti-CCP was 62% while RF was 64% (Table I). Including patients who were positive for either test increased the sensitivity of the serologic tests to 71%. The specificity of anti-CCP was 97% compared to 90% for RF, while it was 99% when both were positive (Table II). Anti-CCP was statistically more specific than RF, while the difference in sensitivity was not significant. Patients who were positive for both serologic markers had a post-test probability of having RA of 86%, while RF yielded a 56% and anti-CCP a 79% post-test probability of having the disease. Likelihood ratios also favored anti-CCP over RF. Very low and very high levels of anti-CCP reactivity were very strongly associated with the absence and presence of RA, respectively.

Table I			Table II		
RA N=132	CCP+	CCP-	Not RA N=636	CCP+	CCP-
	RF+	12		RF+	8
	RF-	10		RF-	14
		38			58
					556

Discussion: This is the first study of anti-CCP in a population selected because an RF test was ordered. Including the anti-CCP results yielded increased diagnostic probabilities that are statistically better than RF alone in both early and established RA patients in this cohort. Thus, anti-CCP is a clinically useful test in the patient population where RF is ordered. 21% of the RF negative RA group, who are often difficult to diagnose, were anti-CCP positive. Only 22 of the non-RA patients were anti-CCP positive. Follow up studies on two groups of patients - those who are not diagnosed with RA but are positive for anti-CCP, and those who are diagnosed with RA but negative for both anti-CCP and RF - will yield further insight into the diagnostic as well as prognostic utility of anti-CCP.

INTRODUCTION

In the 20th century the treatment of RA was conservative. At the beginning of the 21st century this treatment strategy was turned upside down! Early treatment with biologic drugs like TNF-alpha inhibitors and the B-cell depleting anti-CD20 monoclonal antibody can slow progression of RA in over half the patients before irreversible joint damage occurs², but they have side effects and are as expensive as a car.

The anti-CCP ELISA became available at a time when it became important to diagnose RA early in the course of the disease. It is well documented that anti-CCP has higher specificity than RF³, and appears early in the course of disease when some patients are RF negative⁴. RA patients who are positive for anti-CCP or RF have a higher likelihood of progressive disease than those who are negative for these serologic markers⁵. Thus, anti-CCP is a specific marker of early RA that is correlated with disease severity.

However, the true diagnostic power of anti-CCP is not known because the most statistically relevant population, namely the group currently tested for RF in a clinical lab, has never been tested for anti-CCP. It is important to understand the clinical utility of anti-CCP in the group tested for RF, because this is the group that would be tested for anti-CCP if it were added to new criteria to diagnose early RA.

METHODOLOGY

Patients: In this prospective study, informed consent to test for anti-CCP was obtained from 1025 consecutive patients whose doctors ordered an RF test for them. These patients attended primary, secondary and tertiary academic centers from Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil. Inclusion criteria were: be an adult greater than 18 years old, an RF test was ordered and performed, serum was obtained so further testing on anti-CCP was possible, and a definitive diagnosis was obtained within one year from the original testing. In the end, 768 patients met the criteria.

Methods: Igm-RF was determined by nephelometry in a BNII analyzer. Levels > 15 U/mL were considered positive. Anti-CCP antibodies were detected by a second-generation ELISA (INOVA Diagnostics, Inc., San Diego, USA). Levels > 20 IU/mL were considered positive. Both cutoffs were according to manufacturer's instructions. RA was diagnosed based on the ACR criteria.

Statistics: Mann-Whitney and Kruskal-Wallis analyses were used for non-parametric or independent variables. A receiver-operating characteristic curve of anti-CCP and RF at each possible cutoff was drawn⁶. Sensitivities, specificities, positive predictive value (PPV), negative predictive value (NPV), likelihood ratios (LRs), and posttest probabilities (PTP), together with their 95% confidence intervals (95%CI) were computed considering Bayes theorem⁷.

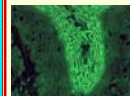
Clinical Sensitivity and Specificity of Anti-CCP Compared with RF

RA N=132			Not RA N=636		
	CCP+	CCP-		CCP+	CCP-
	RF+	12		RF+	8
	RF-	10		RF-	14
		38			58
					556

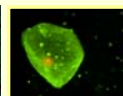
Nearly 80% (82 of 104) of the patients who were positive for anti-CCP have RA, while only 56% (84 of 150) of the RF positive patients have the disease. The sensitivity and specificity of anti-CCP are 62% and 97%, respectively, while those for RF are 64% and 90%.

Immunofluorescence

Anti-Keratin on Rat Esophagus



Anti-Perinuclear Factor on Buccal Cells



Antibodies against citrullinated proteins are found in patients with RA and yield characteristic immunofluorescent patterns on rat esophagus (far left) and human buccal cells (near left)⁸. These are the same antibodies that bind to CCP in the ELISA.

Distribution of Diagnoses in 768 Patients

Clinical Diagnosis	Number of Pts	Percentage
Rheumatoid Arthritis	132	17%
Other Connective Tissue Disorders	102	13%
Spondyloarthropathies	38	5%
Soft Tissue Rheumatism	264	34%
Non-Rheumatologic Autoimmune Disorder	42	5%
Cancer	30	4%
Other Non-Classified Diseases	160	21%

The Pre-test Probability of Disease in This Population is 17%

The average age was 52 +/- 15 years, 79% were women, the average duration of disease was 12 months with a range of 0-720, and 621 (81%) were sent for testing as outpatients, while the rest were inpatients.

Statistical Properties of anti-CCP and Rheumatoid Factor

	Anti-CCP	RF	P Value
Sensitivity	62%	64%	.83
Specificity	97%	90%	<.001
Positive Predictive Value	79%	56%	<.001
Negative Predictive Value	92%	92%	.99
Positive Likelihood Ratio	17.9	6.2	<.001
Negative Likelihood Ratio	.4	.4	.99

Disease Probabilities with Combination anti-CCP and RF

	Likelihood Ratio	*Post-test Probability	Number RA	Number Not RA
Anti-CCP Neg/RF Neg	0.3	6.4%	38	556
Anti-CCP Neg/RF Pos	1.0	17%	12	58
Anti-CCP Pos/RF Neg	3.4	41.3%	10	14
Anti-CCP Pos/RF Pos	43.4	90%	72	8

*Based on the observed pre-test probability of RA of 17%.

CONCLUSIONS

1. Anti-CCP is more useful than RF to help diagnose RA in the population tested for RF. Nearly 80% (82 of 104) of the patients who were positive for anti-CCP have RA, while only 56% (84 of 150) of the RF positive patients have the disease.
2. Anti-CCP is significantly more specific than RF, thus leading to better positive predictive value (79% compared to 56%) and likelihood ratio (17.9 compared to 6.2) than RF. The two tests show the same sensitivity.
3. The combination of the two test results yields higher diagnostic power than either alone. Patients who are double negative have only a 6.4% post-test probability of having RA, while double positive patients have a 90% probability of having RA.
4. Anti-CCP should be included as part of any new criteria to diagnose early RA because anti-CCP has more diagnostic power than RF in the population currently tested for suspicion of RA.

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