

DETECTION OF ANTI-CCP AND DIAGNOSIS OF RHEUMATOID ARTHRITIS

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ABSTRACT

Objective: Develop a convenient enzyme-linked immuno-sorbant assay (ELISA) for the detection of autoantibodies to CCP (Cyclic Citrullinated Peptide) to help diagnose Rheumatoid Arthritis (RA). Compare the results obtained with this new antigen to clinical diagnosis and to the results of tests for IgM and IgG Rheumatoid Factor (RF). Compare the performance of the second generation CCP antigen to the original.

Methods: ELISA plates are coated with a high specific activity cyclic peptide in which the amino acid citrulline is present at key parts of the sequence. Over 1000 clinically defined patients from 4 laboratories were tested for anti-CCP and also IgM and IgG RF.

Results: Only 4 of 316 samples from normal blood donors were positive on the QUANTA Lite™ CCP ELISA. Three samples were low positive while 1 was high positive, a specificity in this group of 99%. Interestingly, the high sample was also a high positive for IgG RF, a specific antibody marker for RA. For patients with RA, the sensitivity of this test was 76%, 193 out of 252 RA patients were positive for anti-CCP. Importantly, 40% of the RA patients who were RF negative were positive for anti-CCP, while 90% of the RF positive RA patients were also positive for anti-CCP. A group of patients with Sjögren's syndrome who had a 75% false positive rate for IgM RF were all negative for anti-CCP. In all rheumatic disease controls there was a 91% specificity for anti-CCP. The second generation peptide is about 20% more sensitive than the first peptide at detecting RA patients, while it is equally as specific.

Conclusion: Anti-CCP antibodies are a very useful new diagnostic marker for RA. Anti-CCP show high sensitivity even in IgM RF negative RA patients, and it shows very high specificity in healthy volunteers and control groups.

METHODOLOGY

The INOVA QUANTA Lite™ CCP ELISA is a semi-quantitative immunoassay that detects autoantibodies against CCP (Cyclic Citrullinated Peptide). These autoantibodies are markers for Rheumatoid Arthritis.

CCP is coated onto each well of a 96 well ELISA plate. Diluted patient sample is incubated in the wells for 30 minutes, followed by a 30 minute incubation with an anti-IgG conjugate. Bound antibodies are quantitated with a colorimetric substrate.

Fig. 1
Anti-Keratin on Rat Esophagus

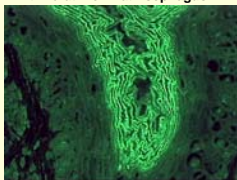
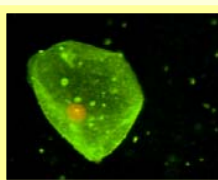


Fig. 2
Anti-Perinuclear Factor



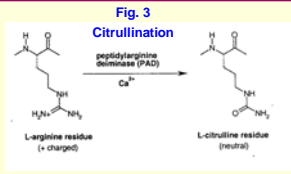
INTRODUCTION

Rheumatoid Arthritis (RA) is one of the most common systemic autoimmune diseases. The etiology of the disease is unknown. Approximately 0.5% of the world population is affected, and twice as many women as men get the disease¹. The diagnosis of RA depends primarily on clinical manifestations of the disease. Until recently, the only serological test routinely used was the determination of the presence of rheumatoid factors (RF) in the serum. RF are antibodies directed to the Fc region of immunoglobulins of the IgG class. RF can be in the IgM, IgG and IgA immunoglobulin classes. The presence of IgM RF is a hallmark of RA, found in approximately 50-90% of these patients^{2,3}. However, IgM RF are found in people with infections, other autoimmune diseases, and some healthy individuals^{1,2}.

It is important for disease management to diagnose and treat people with RA as early as possible³. It has been known for many years that anti-perinuclear autoantibodies, also called anti-keratin, are found in people with RA^{4,5} (see Figures 1 and 2). Recently it was discovered that these antibodies recognize an epitope that contains the deiminated form of arginine called citrulline⁶ (Figure 3). A circular peptide containing citrulline called CCP (Cyclic Citrullinated Peptide) was found to be better at discriminating RA patients from other patients than either the perinuclear antibody test or the test for RF^{7,11}. Additionally, RA patients with anti-CCP may progress to a more severe disease than those who do not have anti-CCP^{10,11}. In published literature, approximately 70% of patients with RA are positive for anti-CCP, while only about 2% of healthy blood donors and disease control subjects are positive. Thus, both negative and positive results have high predictive values¹². A recent study found citrullinated proteins in the joints of patients with RA, but not in joints from healthy volunteers¹³. These results lend a theoretical basis for the presence of anti-CCP in RA patients and a possible pathogenic role for these antibodies.

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Precision and Reproducibility

The within run variation was measured in a number of ways and was very good, averaging 5.6% for a moderately positive sample.

Between run variation was measured by running samples on 6 separate days. The results below are typical.

	Neg1	Low1	Mod1	Mod2	High1	High2
Mean	2 U	21 U	42 U	47 U	103 U	168 U
S.D.	0.1	1.2	2.6	3.1	5.6	12
%C.V.	7%	6%	6%	7%	5%	7%

Comparison with first generation peptide

	# RA Pos.	%Pos RA	%Pos Cts
First generation CCP	36/60	60%	8%
Second Generation CCP	49/60	82%	8%

Clinical Sensitivity and Specificity of the CCP ELISA

Patient Groups	No.	Anti-CCP ELISA No. Pos.	(%)
Blood Donors	316	4*	(1%)
Rheumatoid Arthritis	252	193	(76%)
Rheumatoid Arthritis RF+	183	165	(90%)
Rheumatoid Arthritis RF-	69	28	(40%)
Rheumatic disease controls	336	29*	(9%)
SLE	103	10*	(10%)
Scleroderma	86	10*	(12%)
Sjögren's Syndrome	38	0	(0%)
Other Rheumatic Diseases	109	9*	(8%)
Infectious disease controls	87	2	(2%)
Cryoglobulinemia (75% RF+)	29	2	(7%)
Other controls	29	1	(3%)

*Some of the samples that were positive for CCP antibodies were also positive for IgG RF, another antibody marker of RA. These included 1 healthy volunteer, 2 SLE patients, 4 Scleroderma patients, and 3 of the other rheumatic disease controls.

Linearity using a standard curve instead of a single point

Dil. Factor	O.D.	Units	Units * Dil. Fac
1	1.41	138	138
2	0.79	71	142
4	0.42	31	124

CONCLUSIONS

- The QUANTA Lite™ CCP ELISA is a sensitive and specific autoantibody test to aid in the diagnosis of Rheumatoid Arthritis.
- The CCP ELISA has an overall sensitivity of 76% for RA. It is 90% sensitive for RF+ RA, and 40% sensitive for RF- RA.
- The second generation CCP test is greater than 20% more sensitive than the original test while retaining excellent specificity.
- The specificity compares favorably with other tests. Anti-CCP is 99% specific for healthy volunteers, 91% specific in rheumatic disease controls, and 98% specific in infectious disease controls. This is much better than IgM RF, the autoantibody currently most associated with RA.
- The QUANTA Lite™ CCP ELISA has received 510(k) clearance from the FDA.