Evaluation of a new specific marker for diagnosis of rheumatoid arthritis: the anti-cyclic citrullinated peptide antibody (anti-CCP)

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BACKGROUND

Rheumatoid arthritis (RA) is a common, chronic, systemic autoimmune disease with an estimated prevalence of about 1-2 % of the world-wide population. It is characterized by inflammation of the joints. The diagnosis of RA is based on clinical, radiological and immunological features with rheumatoid factor (RF) being found in 60 – 80 % of patients. But RF is not disease specific as it prevalent in around 15 % of healthy eldery individuals and in patients with other autoimmune diseases and chronic infections. One promising new marker is the anti-CCP antibody which can be measured using an ELISA semi-quantitative assay.

MATERIALS

A retrospective study was conducted using 126 frozen sera.

Groupe	n =	RF pos	RF nég
RA	43	22	21
SLE	19	9	10
HCV pos	20	10	10
Sjögren	23	14	9
MCTD	7	2	5
HyperIg	14	4	10

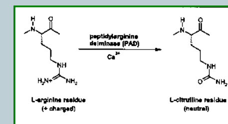
Negative control consist of 15 sera from healthy subjects.

All sera were stocked at – 80 °C and thawed just before analyses were performed.

METHODS

* **Nephelometry**: Rheumatoid factor was measured using a commercial BNII nephelometer analyzer (Dade Behring Switzerland). Polystyrene particles coated with an antigen-antibody complex of human g-globulin/anti-human g-globulin from sheep are agglutinated when mixed with samples containing rheumatoid factors. The intensity of the resulting scattered light is dependent upon the RF content.

* **ELISA technique**. Anti-CCP antibodies were measured using the QUANTA Lite ™ Anti-CCP ELISA (Inova diagnostics, Inc. San Diego, CA; commercialized in Switzerland by Ruwag AG). Pre-diluted controls and diluted (1:101) patient sera were added in duplicate to separate well and incubated for 30 min. at room temperature (RT), allowing any anti-CCP antibodies present to bind to the synthetic cyclic citrullinated peptide bound to the surface of the microwell plate. Unbound sample was washed away and an enzyme labelled anti-human IgG antibody was added to each well and incubated for 30 min. at RT. After washing, the remaining enzyme activity was measured by adding the TMB chromogenic substrate for 30 min. and the optical density (OD) at 450 nm was measured using a microplate reader. The reactivity for each sample was then calculated by dividing the average OD of the sample by the average OD of the CCP ELISA low positive control found on the label. The sera were classified as negative (< 20 Units), weak positive (20 – 30 Units) and moderate to strong positive (> 30 Units).



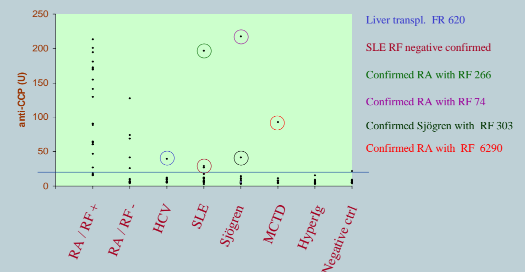
RESULTS :

1. In the 43 RA sera tested, 27 (62.8 %) were anti-CCP positive (Tab.1) . In 83 RA negative patients 7 (8.4 %) were anti-CCP positive (fig.3 and Tab. 2) whereas 39 (46.9 %) were RF positive.
2. The 22 RA patients with positive RF were also anti-CCP positive (fig. 2)
3. In the 21 seronegative RA tested, 5 were anti-CCP positive. 4 of them suffered from a clinically confirmed RA (fig.1)

Tab 1 Anti-CCP results

Group	n =	anti-CCP Pos	anti-CCP nég
RA	43	27	16
Patients without RA	83	7	76
SLE	19	3	16
HCV pos	20	1	19
Sjögren	23	2	21
MCTD	7	1	6
HyperIg	14	0	14
Healthy ctrl.	15	1	14
Total	141	35	106

Fig.3: Anti-CCP positive in patients without RA (n = 7)



Tab.2 Anti-CCP « False positive results »

Group	RF +	Anti-CCP +	Comments
HCV	10	1	
SLE [1]	9	1*	* confirmed RA
Sjögren	14	2*	* 1 conf. RA
MCTD	2	1*	* confirmed RA
HyperIg	4	0	

[1] 2 cases RF- / anti-CCP pos (27 et 29 U)

Fig.1 Anti-CCP in seronegative RA

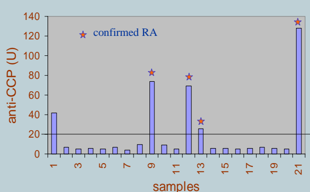
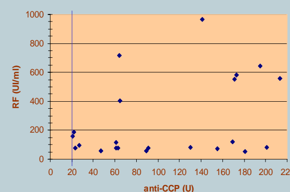


Fig.2 Rheumatoid factor versus anti-CCP antibodies



Anti-CCP	RA +	RA -	
+	30	5	35
-	16	90	106
	46	95	141

Sens. 65 %

Spec. 95 %

PPV : 86 %

NPV: 85 %

CONCLUSIONS :

- The anti-CCP antibody assay is a very useful test for the diagnosis of RA. Since we found a few cases which anti-CCP are present early in the disease, it thus appears that anti-CCP antibodies may have an interesting prognostic value.
- The high specificity of the anti-CCP assay should be helpful in the differential diagnosis of RA and other rheumatic diseases.