ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODIES AND RHEUMATOID FACTOR IN SJÖGREN'S SYNDROME

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Abstract

Objectives: The purpose of this study was to evaluate the prevalence and clinical significance of anti-cyclic citrullinated peptide antibodies (anti-CCP-Abs), IgM and IgA rheumatoid factors (RFs) in primary Sjögren's Syndrome (pSS).

Materials and Methods: We compared clinical and serological characteristics of 31 pSS and 31 Rheumatoid Arthritis (RA) patients. Both, anti-CCP-Abs and RFs (IgM, IgA) directed against Fc determinants of IgG from humans and rabbit were detected by enzyme-linked immunosorbent assay (ELISA). We included 31 blood donors as control group for the evaluation of RFs and anti-CCP-Abs. Nine (29%) pSS patients presented arthritis, and 10 (32,3%) RA patients also had secondary Sjögren's syndrome (sSS)

Results: IgM and IgA RFs prevalence was similar in pSS and RA, whichever the antigene (Human or Rabbit IgG) used. However, RA patients with sSS showed a tendency to present more often RF positivity, longer disease duration and higher ESR and CRP when compared with pSS patients with arthritis. Anti-CCP-Abs were detected in 64,5% of RA patients and in only 6,9% of pSS patients ($p<0,0005$). Anti-CCP-Abs were more often positive in RA patients with sSS (RA/sSS) (8 patients, 80%) than in RA patients without sSS (18 patients, 58,1%), and were absent in pSS patients with arthritis. RF-positive pSS patients presented more often pulmonary involvement and higher inflammatory parameters, and less often neuropathy compared to RF-negative patients. In controls, anti-CCP-Abs were absent and RFs were negligible.

Conclusions: Anti-CCP-Abs were detected in only a few pSS patients, none of whom presented arthritis, which contrasts with the high frequency of these antibodies in RA/sSS. These results suggest that anti-CCP-Abs could be useful in the distinction between pSS and RA with sSS. Although not useful for the differential diagnosis between RA and pSS, RFs may have a prognostic role in pSS.

Keywords: Sjögren's Syndrome; Anti-CCP Antibodies; Rheumatoid Factors; Rheumatoid Arthritis.

Introduction

Primary Sjögren's Syndrome (pSS) is a systemic autoimmune rheumatic disease characterized by lymphocitic infiltration and dysfunction of exocrine glands,[1] causing xerostomia and xerophtalmia. Extraglandular involvement is frequent and includes non-erosive arthritis[2] that may be deforming and present a distribution similar to rheumatoid arthritis (RA), which can lead to difficulties in its discrimination from secondary Sjögren's syndrome (sSS) associated with RA.

Several autoantibodies are associated with pSS,[3] of which the most frequent are anti-SSA, anti-SSB antibodies, and RFs. The detection of RFs in a high percentage of pSS patients could be another obstacle for the distinction between RA and pSS, especially in patients with polyarthritis. The identification of anti-CCP-Abs and its recognition as an important diagnostic tool in RA[4,5] is helpful in the differential diagnosis betwen RA and other rheumatic diseases. However, although highly specific for RA, anti-CCP-Abs have been described in low frequency associated with diseases such as psoriatic arthritis,[6] systemic lupus erythematosus,[7] mixed connective tissue disease,[8] systemic sclerosis,[9] as well as in pSS.[10]

Our purpose was to evaluate the prevalence of anti-CCP-Abs and RFs (anti-Human and Rabbit IgG) of IgM and IgA isotypes in pSS and to establish their correlation with clinical and laboratorial data.

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Materials and Methods

Patients

The study included a total of 93 individuals: 31 pSS patients (all female, with mean age ± SD of 55,6 ± 13,1 years and disease duration of 8,8±7,7 years), 31 RA patients (96,8% [30/31] women, with mean age ± SD of 57,9 ± 12,0 years and disease duration of 10,8 ± 9,2 years). There were 10 RA patients with sSS (32,3%), and 9 pSS patients with non-erosive arthritis (29,0%) (5 with polyarthritis and 4 with oligoarthritis). In pSS patients, mean ESR was 35,8 mm (SD= 20,4), whereas in RA it was 37,1 mm (± 29,3) (p=0,620). Mean CRP was 5,67 g/l (SD=10,2) in pSS and 14,35 g/l (± 20,8) in RA patients (p=0,025). Leukopenia (defined as a leukocyte count bellow 4000 cells/mm^3) was present in 9 patients with pSS (29,0%) and in only 1 RA patient (3,3%) (p=0,007). Systemic involvement, other than sSS in RA and arthritis in pSS, occured in 11 pSS (35,5%) and in 10 RA patients (32,3%).

Sjögren´s syndrome, either primary or secondary, was defined according to the American-European Consensus Group diagnostic criteria,[11] and RA was defined according to the American College of Rheumatology criteria.[12] Thirty one (31) healthy controls (blood donors), matched by age and sex (100% female) were also analized. All patients were followed in a single Rheumatologic Institution. Clinical and epidemiological data were collected retrospectively from each patient.

Anti-CCP Abs and RFs detection

The measurement of anti-CCP-Abs was done using an ELISA kit (Quanta Lite™ CCP3, INOVA Diagnostics, Inc. San Diego, CA), with a cut-off value of 60 U/ml. Commercial ELISA assays were also used to detect IgM-IgA RFs anti-Human IgG (Orgentec Diagnostika GmbH) and anti-Rabbit IgG (Quanta Lite™, INOVA Diagnostics, Inc. San Diego, CA). On the evaluation of RFs anti-Human IgG, serum samples with a value of 50 U/ml were considered positive. For RFs anti- Rabbit IgG, the cut off value was 12 U/ml.

All serum samples were evaluated in duplicate.

A laboratorial screening consisting of complete blood count, CRP and ESR was performed to each patient when colecting the blood samples for the immunological assays.

Statistical analysis

Qualitative values between the groups were compared by the χ^2 test and quantitative values by the non-parametric Mann-Whitney test using SPSS version 13.0 (SPSS Inc., Chicago, IL). Significance was assumed at p<0,05.

Results

The anti-CCP-Abs were positive in 20 RA patients (64,5%), compared with only 2 (6,9%) pSS patients (p<0,0005) and none in the control group (p<0,0005) (Table I). None of the pSS patients with arthritis was anti-CCP-Abs-positive, which contrasts with the RA/sSS patients, of whom 80% presented anti-CCP-Abs positivity (0% vs. 80%; p=0,001) (Table II). In RA patients the presence of sSS was associated with increased prevalence of anti-CCP-Abs when compared with RA patients without sSS (80,0% vs. 57,1%; p= 0,214).

On what concerns to the RFs evaluation, we found the following results: comparing the pSS and RA patients to the healthy controls, we observed that IgM-RFs were not detected in controls and only one blood donor was IgA-RF positive (p<0,0005). Between patients with pSS and RA, there were no significant differences for both IgA and IgM classes of RF using either human IgG or Rabbit IgG as antigen (Table I).

Primary Sjögren's syndrome patients having arthritis were compared with RA patients associated with sSS. RFs of both IgA and IgM classes were more often positive in RA/sSS patients than in pSS/arthritis patients (Table II), being IgM anti-Rabbit IgG RF levels significantly higher (37,5% vs. 90,0%; p=0,019). In adition to a greater RF positivity, RA/sSS patients also tended to be older, present longer disease duration, and higher inflammatory parameters – ESR of 42,0 mm *vs.* 51,1 mm and CRP of 11,0 vs. 3,1 mg/l (p=0,022) – when compared to pSS/arthritis patients.

We evaluated the clinical and laboratory data of pSS patients according to the presence of RFs (IgM, IgA) anti-Human IgG as well as anti-Rabbit IgG (Table III). We found higher levels of inflammatory parameters – ESR and CRP – in RF-positive patients, whichever the antigen used, being particularly significant the association of increased ESR with anti-Human IgG RF positivity (p=0,034 for the IgM RF and p=0,001 for the IgA RF).

Peripheral neuropathy correlated negatively with the presence of IgM RF, either anti-Human IgG (0% vs. 20,0%; p=0,060) or anti-Rabbit IgG (0%

Table I. Prevalence of anti-CCP-Abs and IgM and IgA RFs in patients and controls (χ^2 test)

	pSS	RA	Controls	pSS vs RA	pSS *vs* controls	RA *vs* controls
	n (%)	n (%)	n (%)	p	p	P
IgM RF anti-Human IgG	16 (51,6)	17 (54,8)	0 (0)	0,799	<0,0005	<0,0005
IgA RF anti-Human IgG	17 (54,8)	18 (58,1)	1 (3,2)	0,798	<0,0005	<0,0005
IgM RF anti-Rabbit IgG	16 (51,6)	23 (74,2)	0 (0)	0,123	<0,0005	<0,0005
IgA RF anti-Rabbit IgG	24 (80,0)	23 (74,2)	2 (6,5)	0,590	<0,0005	<0,0005
Anti-CCP-Abs	2 (6,9)	20 (64,5)	0 (0)	<0,0005	0,137	<0,0005

vs. 23,1%; p=0,042), whereas IgA RF did not appear to have any influence. Pulmonary involvement was more frequent in RF-positive pSS patients compared with RF-negative patients, with IgA-RF anti-Human IgG achieving statistical significance (29,4% vs. 0%; p=0,027).

Interestingly, we did not observe in pSS patients any association between the presence of arthritis and RFs specific for human and animal antigens.

Discussion

In clinical practice the distinction between pSS and RA is of great importance, and since both diseases share clinical and serological characteristics, reliable diagnostic tools are needed.

Our population of pSS had clinical and laboratory characteristics similar to other published series of patients with pSS.[13]

We found anti-CCP Abs in 6,9% pSS patients. Our data are in agreement to other authors who also detected these antibodies in pSS. Goëb et al[14] found anti-CCP Abs in only 4% of 137 women and 18% of 11 men with pSS. Gottenberg et al[10] in a cohort of 134 patients with pSS found that 7,5% of the samples were positive for anti-CCP Abs. Atzeni et al[15] recently reported that 10% of 141 individuals with pSS were positive for those Abs. We have to point out that none of our patients with anti-CCP Abs had arthritis. Maybe in the future these patients will develop RA. It is described that these antibodies can appear some years before the development of RA symptoms (Nielen et al).[16] Atzeni et al[15] also suggested that anti-CCP positivity in patients with pSS may be a predictor of future progress to RA.

Anti-CCP Abs were detected in 64,5% of RA patients. The differences between RA patients and the other groups were highly significant (p< 0,0005 vs pSS; p< 0,0005 vs controls). Our data are in agreement to other authors[4,5] who demonstrated that anti-CCP Abs are specific markers for RA. In RA patients, anti-CCP-Abs were more frequent in those with sSS than in those without sSS, however the difference was not statisticaly significant. We do not know if this result would be the same if we had a higher sample.

As expected, RFs of both isotypes were significantly more frequent in patients than in controls. On the other hand, there were no significant differences of IgM and IgA RFs between pSS and RA patients. However, IgM anti-Rabbit IgG RF was more frequently detected in RA than in pSS (74,2 vs 55,2%), although not reaching statistical significance (p=0,123). There are authors

Table II. Comparative analysis of patients with pSS/arthritis and RA/sSS

	pSS/Arthritis (n=9)	RA/sSS (n=10)	p
Age (years)	56,3±10,8	62,0±8,8	0,243
Evolution (years)	9,2±7,3	13,8±12,5	0,541
ESR (mm)	42,0±27,4	51,1±31,4	0,829
CRP (mg/l)	3,1±3,2	11,0±11,2	0,022
Leukopenia	2 (22,2%)	1 (11,1%)	0,527
IgM RF anti-Human IgG	4 (44,4%)	6 (60%)	0,498
IgA RF anti-Human IgG	5 (55,6%)	7 (70%)	0,515
IgM RF anti-Rabbit IgG	3 (37,5%)	9 (90%)	0,019
IgA RF anti-Rabbit IgG	7 (87,5%)	9 (90%)	0,867
Anti-CCP-Ab	0 (0%)	8 (80%)	0,001

Table III. Comparative analysis of clinical and laboratorial features in pSS patients, according with the positivity for IgM and IgA RFs.

RF	IgM RF anti-Human IgG n(%)			IgA RF anti-Human IgG n(%)			IgM RF anti-Rabbit IgG n(%)			IgA RF anti-Rabbit IgG n(%)		
Features	**+ (n=16)**	**– (n=15)**	**p**	**+ (n=17)**	**– (n=14)**	**p**	**+ (n=16)**	**– (n=13)**	**p**	**+ (n=24)**	**– (n=6)**	**p**
Age (years) (mean±SD)	54,0±15,3	57,4±10,4	0,476	53,8±14,1	57,9±11,8	0,393	53,7±14,9	59,2±10,5	0,210	57,3±13,5	51,7±10,6	0,299
Evolution (years) (mean±SD)	8,6±8,7	9,0±6,6	0,625	8,8±8,7	8,7±6,2	0,723	8,5±9,1	9,8±6,3	0,263	9,0±8,1	9,0±7,0	0,900
ESR (mm) (mean±SD)	43,1±21,4	27,6±16,1	0,034	46,2±19,7	22,3±11,5	0,001	39,8±23,3	28,9±16,2	0,210	38,6±21,9	24,7±10,7	0,125
CRP (mg/l) (mean±SD)	8,4±13,7	2,8±1,97	0,873	8,0±13,3	2,8±2,0	0,780	8,0±13,7	3,4±2,9	0,627	6,4±11,4	3,3±2,8	0,464
Parotid enlargement	3 (20,0)	2 (13,0)	0,624	3 (18,8)	2 (14,3)	0,743	3 (18,8)	2 (15,4)	0,811	3 (12,5)	2 (33,3)	0,221
Arthritis	4 (25,0)	5 (33,3)	0,609	5 (29,4)	4 (28,6)	0,959	3 (18,8)	5 (38,5)	0,238	7 (29,2)	1 (16,7)	0,536
Raynaud's phenomenon	5 (31,3)	5 (33,3)	0,901	5 (29,4)	5 (35,7)	0,709	5 (31,3)	4 (30,8)	0,978	7 (29,2)	2 (33,3)	0,842
Systemic involvement	8 (50)	9 (60,0)	0,576	10 (58,8)	7 (50)	0,623	8 (50)	8 (61,5)	0,534	13 (54,2)	3 (50)	0,855
Skin	1 (6,3)	3 (20,0)	0,254	2 (11,8)	2 (14,3)	0,835	2 (12,5)	2 (15,4)	0,823	4 (16,7)	0 (0)	0,283
Neuropathy	0 (0)	3 (20,0)	0,060	1 (5,9)	2 (14,3)	0,431	0 (0)	3 (23,1)	0,042	2 (8,3)	1 (16,7)	0,543
Lung	4 (25,0)	1 (6,7)	0,165	5 (29,4)	0 (0)	0,027	3 (18,8)	2 (15,4)	0,811	5 (20,8)	0 (0)	0,221
Liver	1 (6,3)	1 (6,3)	0,962	1 (5,9)	1 (7,1)	0,887	1 (6,3)	1 (7,7)	0,879	1 (4,2)	1 (16,7)	0,272
Leukopenia	5 (31,3)	4 (26,7)	0,779	5 (29,4)	4 (28,6)	0,959	5 (31,3)	3 (23,1)	0,624	7 (29,2)	1 (16,7)	0,536

who reported that Rabbit IgG is more specific for RA.[17-20] Once again, we think the size of our sample may have contributed to this result.

When we analyzed the clinical and laboratory data in patients with RA/sSS and pSS/arthritis, we found that RA/sSS patients were older and had higher ESR and CRP. The RFs of IgM and IgA isotypes were also more prevalent in AR/sSS patients than in pSS/arthritis, particularly IgM anti-Rabbit IgG RFs (90,0 vs 75,5%; p=0,022). This result is also in agreement to what we have mencioned above, RFs anti-Rabbit IgG are more specific for RA.

Although RFs of either IgM or IgA isotypes are not a reliable diagnostic tool to differenciate pSS and RA, they do seem to have clinical implications in pSS.[21] Inflammatory parameters, namely ESR and CRP were higher in RF-positive patients, with IgM and IgA anti-Human IgG RF-positive pSS patients having significantly higher ESR than RF-negative pSS patients. RF-positive pSS patients had more frequently pulmonary involvement than RF-negative patients, although statistical significance was only found in the positivity to IgA anti-Human IgG RF (29,4 vs 0%; p=0,027). Lung involvement in pSS was also reported by Atzeni et al[15] in 13% of patients with pSS. However, they did not find any correlation between RF titers and extra-glandular involvement. This discrepancy may be explained by the fact we used different methods for RF detection. The immunonephelometry does not detected IgA RFs.

In our study, RF-positive pSS patients presented less frequently peripheral neuropathy, although only 3 cases were reported. Neuropathy was particularly less frequent in IgM RF-positive patients, either anti-Rabbit-IgG (0 vs 23,1%; p=0,042) or anti-Human IgG (0 vs 20,0%; p=0,060). It is noteworthy to refer that RF positivity in pSS patients was not related with the presence of arthritis.

As already mencioned, the differential diagnosis between pSS and RA is very important. The results of this study showed that anti-CCP-Abs were rarely present in pSS patients. This serological marker seems to help to descriminate between pSS patients and RA patients with sSS. We can not rule out the possibility that pSS patients with anti-CCP Abs may develop RA, especially in patients who are also positive for IgM and/or IgA RFs. These patients must have a clinical and radiographic follow up. Our study also suggests that, as occurs in RA,[22] RFs can have a prognostic role in pSS. Although the small size of our sample, our results are comparable to results found by other authors.[10,14,15]

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