Anti-cyclic citrullinated peptide antibody determination in synovial fluid of psoriatic arthritis

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Abstract

Objective
To assess the role of anti-CCP antibodies in synovial fluid (SF) of psoriatic arthritis (PsA) patients by analysing their association with different clinical patterns of the disease

Methods
Seventy-five patients with a knee-joint effusion were studied, including 31 PsA patients, 29 rheumatoid arthritis (RA) and 15 osteoarthritis (OA) patients. SF and paired serum samples were stored at -70°C until IgG anti-CCP and total IgG determination. The pattern of PsA articular involvement was defined as mono-, oligo-, polyarticular or axial.

Results
Lower levels of IgG anti-CCP antibodies in SF (p < 0.01) and serum (p < 0.005) were found in PsA respect to RA patients without difference with OA. We found a higher SF/serum ratio for anti-CCP compared to the SF/serum ratio for total IgG in PsA (p < 0.0005) as well as in RA and OA. The correction of anti-CCP concentration in SF as IgG anti-CCP (unit) / total IgG revealed lower (p < 0.002) values in PsA patients with respect to RA patients. In PsA group, values of anti-CCP antibodies, SF/serum ratio of anti-CCP and anti-CCP/IgG above the cut-off were found in 5, 6 and 2 SF samples respectively. The presence or absence of anti-CCP antibodies did not discriminate a particular clinical subset.

Conclusions
In conclusion, strengthening the concept of local production of anti-CCP antibodies within the joint space, our results suggest that anti-CCP antibody detection in SF should take into account corrections such as total amount of corresponding immunoglobulin or SF/serum ratio. In our study, the presence or absence of anti-CCP antibodies did not discriminate a particular clinical subset, but further longitudinal studies are required to clarify the clinical role of anti-CCP in PsA.

Key words
Anti-CCP, synovial fluid, psoriatic arthritis.

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Introduction

The anti-filaggrin antibodies represent a related group of rheumatoid arthritis (RA) specific antibodies first described as perinuclear factor (1). Filaggrin citrullination is an essential step for its immunogenicity (2) and cyclic citrullinated peptides (CCP) are used as antigens in ELISA to detect the corresponding antibodies with sensitivities ranging from 14.4% to 96%, and specificities from 88.9% to 100% (3). Anti-CCP antibodies have been detected in the early phase of RA and have been associated with severe radiological damage (2). A clinical prediction model, discriminating between self-limiting persistent non-erosive and persistent erosive arthritis, also includes the evaluation of anti-CCP antibodies (4). Interestingly, citrullinated fibrin has been identified as one of the major citrullinated proteins in RA synovium (5, 6) and anti-filaggrin (7), antikeratin antibodies (8, 9), or anti-CCP (10) antibodies have been detected in the synovial fluid (SF) of RA patients. Despite reports of the high specificity of anti-CCP test, these antibodies have recently been detected in the serum (11-15) of patients with psoriatic arthritis (PsA), suggesting relevant considerations about the correct diagnosis of this disease. Anti-CCP determination in SF was performed only in a small number of PsA patients, without analysing the clinical role of these antibodies (15). Moreover, an adequate interpretation, considering concurrent evaluation of the corresponding SF immunoglobulin (10) or serum anti-CCP levels (16) of SF anti-CCP was not investigated.

The aim of this study was to assess the role of anti-CCP antibodies in the SF of PsA patients by analysing their association with different clinical patterns of the disease.

Materials and methods

Patients and characteristics

Seventy-five patients with a knee-joint effusion including 31 PsA patients, classified according to Moll and Wright criteria, modified by Helliwell (17), 29 RA patients classified according to ACR criteria (18), and 15 (OA) patients were studied. All patients underwent a careful evaluation of clinical, routine radiographic and laboratory features. SF, obtained by therapeutic arthrocentesis, and paired serum samples were stored at -70 °C until IgG anti-CCP and total IgG were measured.

Clinical evaluation included physical examination of joints for tenderness (n = 68) and swelling (n = 66). The pattern of articular involvement was defined as mono-, oligo- (*i.e.* < 5 involved joints), polyarticular (*i.e.* ≥ 5 involved joints) or axial (inflammatory spinal pain, alternating buttock pain or radiographic evidence of sacroiliitis according to European Spondyloarthropathy Study Group criteria (19). Symmetrical involvement was considered when bilateral involvement > 50% of affected joint area was present (20). A radiographic evaluation of the involved joints was performed.

Routine laboratory investigations included analysis of SF, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Rheumatoid factor (RF) (N latex RF, IU/ml^{-1}) and IgG (g /L^{-1}) determinations were performed using a nephelometry method (Behring, Marburg, Germany).

IgG anti-CCP antibody levels (units) were determined by ELISA (Quanta Lite™CCP IgG ELISA, – INOVA Diagnostics, San Diego, CA) according to the manufacturer's instructions.

Briefly, 100μl of anti-CCP standards and patient samples (diluted 1:100) were added to the wells coated with a purified synthetic CCP antigen. After incubation for 30 minutes r/t the wells were washed 3 times with 200 μl of buffer containing Tris-buffered saline and Tween 20. The microplates were then incubated for 30 minutes r/t with 100 μl of peroxidase-IgG conjugate (goat) anti-human IgG and washed 3 times. A tetramethylbenzidine chromogen substrate solution was added to each well. After 30 minutes r/t the reaction was stopped with solution containing 0.344M sulphuric acid. The adsorbance was read at 450 nm. Positive IgG anti-CCP antibody sample was defined as ≥ 20 units in serum, according to the manufacturer's recommendations, and as > mean +2 standard deviation of 15 OA samples in SF.

Statistical analysis

Categorical variables were analysed by χ^2 test or Fisher's exact test. The results were presented as median (25th – 75th percentile) and the significance of the differences determined using the Mann-Whitney test for unpaired samples and Wilcoxon's test for paired samples. The significance of any correlation was determined by Spearman's rank correlation coefficient; *p* values less than 0.05 were considered statistically significant.

Results

We found anti-CCP antibodies in 6.45% (2/31) of PsA sera. In sera of RA patients anti-CCP antibodies and RF were present in the same amounts (55.2%; n = 16/29). Sixty-two per cent (18/29) of RA patient's sera were positive for anti-CCP antibodies or rheumatoid factor (RF). OA patients did not show anti-CCP or RF serum levels above the cut-off. The main demographic, clinical and laboratory features of PsA, RA and OA patients are shown in Table I. The IgG anti-CCP concentrations in SF and sera of PsA, RA and OA patients are shown in Figure 1. Lower levels of IgG anti-CCP antibodies in SF (*p* < 0.01), and serum (*p* < 0.005) were found in PsA respect to RA patients without difference with OA (Fig. 1). We found a higher SF/serum ratio (median/25th–75th percentile) for anti-CCP compared to the SF/serum ratio for total IgG in PsA (1/0.7-0.4 *vs*. 0.63/0.36-0.93; *p* < 0.0005) as well as in RA (0.97/0.6-1.1 *vs* 0.54/0.44-0.7; *p* < 0.0005) and OA (0.81/0.75-0.99 *vs*. 0.43/0.36-0.6; *p* < 0.001).

The correction of anti-CCP concentration in SF as IgG anti-CCP (unit) / total IgG (g L^{-1}) revealed lower (*p* < 0.002) values (median/25th –75th percentile) in PsA patients (0.65/0.4-1.9) with respect to RA patients (3.24/0.79-21.5). No difference was found in PsA patients with respect to OA (1.1/1.0-1.6).

In the SF of the OA control group, the value above mean +2 standard deviations was 11.9 units for anti-CCP antibodies, 1.53 for SF/serum ratio of anti-CCP and 3.78 unit/g L^{-1} for anti-CCP /IgG.

In the PsA group, values of anti-CCP

Table I. Main demographic, clinical and laboratory features of patients with PsA, RA and OA.

	PsA	RA	OA
Patients (number)	31	29	15
Age (years, mean/range)	47/16-74	53/26-67	59/ 45-78
Males/females (number)	20/11	8/21	5/10
Disease duration (months, mean/range)	99/1-492	109/6-588	86/12-240
Disease onset (years, mean/range)	41/3-64	44/18-72	54/44-66
Tender joint count (0-68)	5/2-18	12/7-26	1/1-4
Swollen joint count (0-66)	2/1-12	8/2-16	1/1-2
DMARDs % (*n*)	29 (9)	83 (24)	0 (0)
Corticosteroids % (*n*)	29 (9)	99 (26)	0 (0)
Serum Anti-CCP +ve % (*n*)	6.4 (2)	55 (16)	0 (0)
Serum RF +ve % (*n*)	6.4 (2)	55 (16)	0 (0)
ESR (mm/1 h)*	20/7-35	36/18-50	12/5-20
CRP (mg dl^{-1})*	1.2/0.3-2.4	1.2/0.6-3.6	0.3/0.2-0.4
SF low viscosity % (*n*)	84 (26)	90 (26)	26.7 (4)
SF Good	22.6 (7)	17.2 (5)	86.7 (13)
Mucin clot Fair	64.5 (20)	55.2 (16)	13.3 (2)
% (n) Poor	12.9 (4)	27.6 (8)	–
SF WBC /mmc*	7000/5400-10000	8000/6000-18075	600/300-1000
SF PMN cells/mmc*	4680/990-7500	6000/4015-14235	120/20-210
SF mononuclear cells/mmc*	2500/1710-3150	2210/1800-3800	570/200-800

*Median/25th–75th percentile.

antibodies, SF/serum ratio of anti-CCP and anti-CCP / IgG above the cut-off were found in 5 (16%), 6 (19%) and 2 (6.4%) SF samples respectively. The main features of patients positive for serum anti-CCP, SF anti-CCP, SF/serum ratio of anti-CCP or SF IgG anti-CCP/total IgG are summarized in Table II. The presence or absence of anti-

CCP antibodies did not discriminate a particular clinical subset. Besides, in two patients with PsA associated to RF and anti-CCP positivity, one had clinical and radiography enthesopathy and the other erosive damage of metathar-sophalangeal joints.

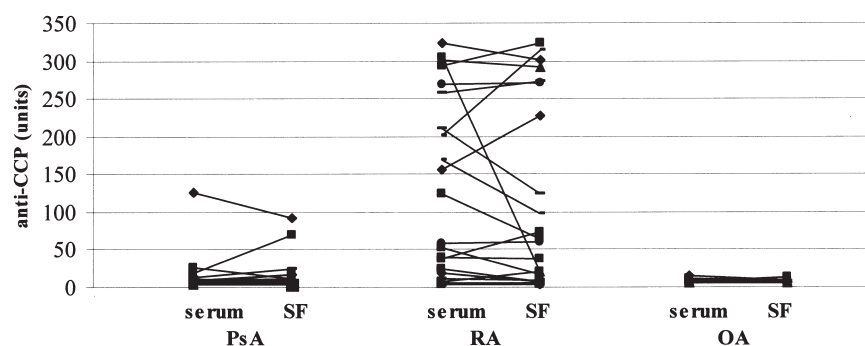Considering that anti-CCP antibodies are related to RA erosive changes, we

Fig. 1. Values of IgG anti-CCP antibodies in serum and SF of PsA, RA and OA patients. Lower levels (median/25th-75th percentile) of IgG anti-CCP antibodies in SF were found in PsA (6/4.8-11.5 units) respect to RA (21/6-124 units) patients (*p* < 0.01), without difference with OA (7.1/6.2-8.1 units). Lower levels of IgG anti-CCP antibodies in serum were found in PsA (6.5/4.9-9.7 units) respect to RA (38/5-202 units) patients (*p* < 0.005), without difference with OA (7.7/7.1-9.9 units).

Table II. Clinical features of PsA patients with anti-CCP antibodies.

Sex	Age (yrs)	Disease duration (months)	Disease onset (yrs)	Serum anti-CCP (unit)*	SF anti-CCP (unit)*	SF Anti-CCP/IgG (g/L⁻¹)*	Anti-CCP SF/serum	RF	Rx changes	Joint pattern	PsA features	DMARD	Psoriasis	HLA typing
M	58	372	28	127	91	66.1	0.70	P**	erosions	poli		no	vulgaris	A26, A29,B14, B27,Cw03, Cw08
F	24	4	24	26	11	1.18	0.45	N	no	oligo axial	dactylitis IBP, asymm.	no	vulgaris, familial	n.a
M	48	144	36	7	17	0.58	2.40	N	erosions ankylosis	poli		INF	vulgaris	n.a
F	49	2	49	13	25	1.93	1.90	N	no	oligo		no	vulgaris, familial	A02,B18, B51,Cw7, Cw16, DRB1*03, DRB1*04
M	74	120	64	10	16	3.92	1.60	P	enthesopathy	poli axial	enthesitis	no	vulgaris	A02, A11,B35, B39,Cw04, Cw12 DRB1*14, DRB1*16
M	30	48	26	19	70	3.57	3.70	N	no	poli	asymmetrical	HCQ	guttate	A10,A19,B05,B35,Cw04 DRB1*13, DRB1*16
M	58	1	57	5	10	0.5	2.1	N	grade 2 sacroiliitis	poli axial	sacroiliitis	MTX	vulgaris	A01,A26, B18,B35,Cw04 DRB1*11, DRB1*16
M	33	12	32	4	11	0.83	2.90	N	no	mono	asymmetrical	no	vulgaris	n.a.

*Values above cut-off in bold; **HCV Ab positive without cryoglobulin; IFN: infliximab; MTX: methotrexate; HCQ: hydroxicloroquine.

hypothesized a correlation between SF anti-CCP antibodies and joint damage in PsA patients, but our statistical analysis did not confirm it. Moreover, we did not find any correlation between SF anti-CCP levels and the main clinical or laboratory parameters, including SF analysis.

Discussion

The clinical picture of PsA shares features of spondyloarthropathies and RA, but asymmetrical oligoarticular disease predominantly of the lower limbs, distal interphalangeal joint (DIP) involvement, enthesitis, dactylitis and typical radiological features are helpful to distinguish it from RA (21). Several autoantibodies are detectable in the serum of RA patients; these include a few RA associated, but not RA specific, antibodies, as well as RA specific autoantibodies, such as anti-CCP antibodies. These seem to be present exclusively in RA (22), and the anti-CCP assay is able to detect a heterogeneous population of RA autoantibodies; they are extremely RA specific with a reasonable sensitivity and a good prognostic value (22). Nevertheless, serum anti-CCP antibodies may be present in PsA patients with a percentage ranging from 7% (14) to 15.7% (13). Although our study considered a short sample of PsA patients, we found a similar prevalence of anti-CCP antibodies (6.4%). We found that IgG anti-CCP antibodies are present in the SF of PsA patients at not significantly different levels with respect to serum levels according to a previous report (15). Nevertheless, the different levels of SF immunoglobulins, including autoantibodies, among arthritic patients and healthy subjects must take into account the potential factors of protein concentrations in SF (23). Thus, adequate interpretation of SF protein levels needs concurrent evaluation of serum concentration and the kinetic factors involved in local blood supply and lymphatic drainage, which could be different for each joint (23). An approach to normalization of the protein serum concentration and the permeability of synovial membrane is to calculate the SF/serum ratio (16). Another possibility is the expression of

autoantibody (*i.e.*, anti-CCP antibodies) concentrations corrected for the total amount of corresponding class-specific immunoglobulin (10). In our study, both approaches sustained a preferential production of these autoantibodies in the joint of RA patients with serum anti-CCP, according to previous reports on antikeratin antibodies (8, 9), as well as anti-CCP antibodies (10). In fact, we found SF/serum ratio for anti-CCP antibody higher than for IgG, confirmed by the evidence that the IgG anti-CCP antibody concentrations, corrected for the total amount of the corresponding class-specific immunoglobulin, was higher in SF than in serum of the RA patients. These results disagree with another report showing equal titers of anti-filaggrin antibodies in serum and SF, but in this study correction of the autoantibody titer, taking into account the total amount of IgG, was not carried out (7). The local production of anti-CCP antibodies, sustained by elevated SF/serum ratio of anti-CCP, or anti-CCP/IgG values in SF, was found in a small proportion of our PsA patients, raising the question of whether positivity for anti-CCP antibodies is caused by the co-occurrence of RA arthritis and psoriasis in such patients. In the inflamed synovium in fact the presence of citrullinated proteins is not specific for RA, but the induction of autoantibodies directed to these proteins is a more specific phenomenon, detectable only in human RA patients (10, 24). In our PsA patients, anti-CCP antibodies were associated in 5 cases with typical features of PsA (Table III), without discriminating the different subsets (*i.e.*, axial or peripheral involvement).

In two patients anti-CCP were associated with RF positivity, a feature considered as an exclusion criteria for PsA by Moll and Wright. Nevertheless, the current classification criteria are debated and Gladman (25) sustained that there is no reason to insist on seronegativity for RF as this factor is found to be positive in people unaffected by arthritis. One PsA patient with anti-CCP antibodies had anti HCV, along with RF, but it has been described that this association does not seem related to HCV infection (26).

Another interesting aspect is the observation that PsA patients with anti-CCP antibodies seem to be associated with a larger number of eroded joints (13). We did not demonstrate this association, but in our study, this finding was observed in 2 PsA patients with local production of anti-CCP antibodies, both showing a symmetric poliarthritis without typical PsA features, suggesting the co-occurrence of RA and psoriasis. Since peripheral symmetric and erosive joint involvement tend to increase over time (27, 28), it is possible that anti-CCP positive patients with mono- or oligoarthritis may develop these features during the course of the disease. Thus an important aspect of our study is the evidence that SF analysis, including the detection of anti-CCP, does not reveal different patterns among patients with PsA.

In conclusion, strengthening the concept of local production of anti-CCP antibodies within the joint space, our results suggest that anti-CCP antibody detection in SF should take into account corrections such as total amount of corresponding immunoglobulin or SF/serum ratio. In our study, the presence or absence of anti-CCP antibodies did not discriminate a particular clinical subset, but further longitudinal studies are required to clarify the clinical role of anti-CCP detection in the classification, diagnosis and monitoring of PsA patients, particularly in selected cases, *i.e.*, seronegative patients for anti-CCP with atypical clinical features of PsA or RA.

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