Anti-cyclic citrullinated peptide and anti-keratin antibodies in patients with idiopathic inflammatory myopathy

Moisés Labrador-Horrillo1, Mª Angeles Martinez2, Albert Selva-O’Callaghan1, Juan Francisco Delgado2, Xavier Martínez-Gómez3, Ernesto Trallero-Araguás1, Jose Luis Rodriguez-Sanchez2 and Miquel Vilardell-Tarrés1

Objective. To investigate the prevalence of anti-cyclic citrullinated peptide (anti-CCP) and anti-keratin antibodies (AKAs) in a cohort of patients with idiopathic inflammatory myopathy.

Methods. In a cross-sectional study, we determined the presence of anti-CCP and AKAs by ELISA and IIF, respectively, in a cohort of 90 consecutive patients with idiopathic inflammatory myopathy. Associations between anti-CCP and clinical manifestations or other autoantibodies were determined with the chi-square and Mann–Whitney U-tests. Radiographs of hands were retrospectively evaluated. Serum autoantibody profile was determined in all patients.

Results. Twelve patients were positive to anti-CCP (13.3%); in eight cases values were moderate–high. AKAs were not detected in any patient. Comparison between patients positive and negative to anti-CCP did not show clinical or biological differences. Arthritis joint erosions or positive status to anti-synthetase antibodies were not more frequent in patients with anti-CCP antibodies. Prevalence of RF was the only variable significantly associated with the presence of these antibodies (P = 0.043).

Conclusions. High titres of anti-CCP can occasionally be found in patients with inflammatory myopathy. Therefore, a possible diagnosis of RA should be considered with caution in these patients.

Key words: Anti-cyclic citrullinated peptide, Anti-keratin antibodies, Idiopathic inflammatory myopathy, Polymyositis, Inclusion body myositis, Arthritis, Myositis-specific antibodies, Autoimmunity, Rheumatoid arthritis, Rheumatoid factor.

Introduction

Anti-cyclic citrullinated peptide (anti-CCP) antibodies are an effective marker for the diagnosis of RA [1, 2]. The so-called anti-keratin antibodies (AKAs), which recognize the protein filamentin in rat oesophageal mucosal cells, have a diagnostic specificity comparable with anti-CCP, but a low sensitivity (46%), which limits their usefulness in diagnosing RA [3]. Both anti-CCP and AKAs specifically bind to substrates containing the modified amino acid citrulline, generated by post-translational modification of arginine residues by the enzyme, peptidyl arginine deaminase [4, 5].

Anti-CCP-positive sera from RA patients are not reactive with control peptides in which citrulline is replaced by another amino acid, indicating that the citrulline moiety is the main antigenic determinant recognized by such sera [6]. Numerous studies have corroborated the high specificity (>95%) of the anti-CCP assay for the diagnosis of RA [1, 2]. The control sera used to assess the discriminative characteristics of this assay mainly came from normal individuals, patients with infectious diseases or cohorts of patients with a large variety of rheumatic diseases [7]. In patients with idiopathic inflammatory myopathy, anti-CCP antibodies are occasionally reported at low titres [8]. Nevertheless, to our knowledge, there are no studies that specifically address the relevance of these antibodies in myositis patients. The aim of this study was to investigate the prevalence and clinical significance of anti-CCP and AKAs in a cohort of patients with idiopathic inflammatory myopathy.

Patients and methods

Patient population

This study included 90 consecutive adult patients with idiopathic inflammatory myopathy receiving care at Vall d’Hebron General Hospital in Barcelona, Spain, between 1983 and 2008. Vall d’Hebron is a 700-bed referral teaching hospital for a catchment population of nearly 450 000 inhabitants. Virtually all patients from the area with suspected myositis are referred to Vall d’Hebron, where they are diagnosed, treated and followed up, whatever the severity of the disease.

The diagnosis of PM/DM was based on the criteria of Bohan and Peter [9, 10]: symmetrical proximal muscle weakness, increased serum muscle enzymes, electromyography abnormalities, typical histological findings on muscle biopsy, and characteristic dermatological manifestations (heliotrope rash and Gottron’s papules). All the patients included met the criteria for definite DM/PM. Patients with idiopathic inflammatory myopathy who met the criteria for another defined CTD were included as patients with myositis overlap syndrome, and those with a diagnosis of cancer within 3 years of the myositis diagnosis were included as patients with cancer-associated myositis. Characteristic clinical and histologic features provided the diagnosis of IBM according to established criteria [11, 12].

Clinical data were obtained by performing a standardized history and physical examination, and by reviewing the patients’ medical records. Blood was drawn into a serum tube from an antecubital vein. All serum samples were aliquoted within 5 h following collection, stored at −80°C, and thawed only once before assay with the commercial kits used. Patients included in the study gave informed oral consent to the use of their serum for research purposes. The study was approved by the institutional review board of our hospital.

Determination of antibodies

Serum samples from each patient were screened by IIF for ANAs using HEp-2 cells, and by ELISA for antibodies against ENAs.
(Ro, La, RNP and Sm) and anti-his-tRNA synthetase (anti-Jo-1). Sera from all patients in this series were tested by protein and RNA immunoprecipitation, which allowed detection of other synthetases, as well as myositis-specific (anti-Mi-2 and anti-SRP) and myositis-associated antibodies (anti-Ro52, anti Ro 60, anti-La, anti-PM/Scl and anti-RNP) that may have been overlooked by ELISA, and confirmed the ELISA results, as previously reported [13].

AKA IgG were determined by two senior immunologists. A semi-quantitative IIF method was used for specific detection and titration of AKA, as described previously [14]. Briefly, AKAs were detected using cryostatic sections obtained from the middle third of rat (Rattus sp.) oesophagus (made in-house). Serum samples and positive and negative controls, both diluted to 1:5 in phosphate buffered saline (PBS, pH 7.2), were applied to the tissue and incubated at room temperature for 30 min in a moist chamber. After two washings, fluorescein-conjugated rabbit anti-human IgG (H+L) (Dako, Glostrup, Denmark) was added and incubated for 30 min; then the specimens were washed and mounted. AKA-positive serum gave distinct laminar or speckled fluorescent staining of the superficial layer (stratum corneum) of rat oesophagus epithelium. Serum samples with positive fluorescence were subsequently titrated.

Anti-CCP antibodies were determined by a second-generation anti-CCP assay (CCP2), using a commercial ELISA (Axis-Shield Corp., Dundee, UK), according to the manufacturer’s instructions. Serum samples from patients were diluted to 1:100 and were considered positive if the antibody titre was >5 arbitrary units, as determined by the dilution of a positive serum standard. Sera with ≤5 arbitrary units were considered negative for anti-CCP, according to the manufacturer’s criteria.

RF IgM was measured according to the standard procedure of the laboratory using a Behring BN II nephelometer (Dade Behring, Deerfield, IL, USA). This method uses human and sheep IgG coated on latex beads as target antigens, and primarily detects RF IgM. The upper limit of the reference range is 12 IU/ml.

**Radiographic measurements**

Radiographs of the hands were taken in all patients with arthritis during follow-up and were retrospectively reviewed by two senior physicians. Radiographs of the joints were available for 78 out of 90 myositis patients.

**Statistical analysis**

The chi-square test (with Yates’s correction, when appropriate) and the Mann–Whitney U-test were used to determine the significance of differences in baseline variables between anti-CCP-positive and -negative patients. All tests were two-sided, and probability (P) values of <0.05 were considered significant.

**Results**

Between 1983 and 2008, 90 patients (73 women, 17 men) with a mean (s.d.) age of 49.16 (16.55) years were diagnosed with inflammatory myopathy in our department. Median follow-up time of the cohort was 6.1 years (9.7 years). Among the 90 patients diagnosed with inflammatory myopathy, 12 (13.3%) were positive to anti-CCP. In four of these patients, anti-CCP reactivity was weak, but in the other eight patients, values were moderate or high (Fig. 1). Data from patients with and without anti-CCP were statistically analysed. None of the patients met the ACR criteria for RA [15] at the onset of their symptoms or during the follow-up of the cohort.

Demographic factors, including age and disease duration, were not significantly different between anti-CCP-positive and -negative patients. None of the patients studied was positive to AKAs. No association was found when other significant variables were analysed (Table 1). Arthritis or positive status to anti-synthetase antibodies was not more frequent in patients positive to anti-CCP antibodies. Joint erosions were not found in any of the myositis patients with arthritis. Radiographs of the hands were available in all the anti-CCP-positive patients. Mean follow-up of the 56 patients who developed arthritis was not different from that of patients without arthritis. The most prevalent type of articular involvement was small symmetrical polyarthritis of the hands and wrists (35/56; 62%); the knees were also occasionally affected. The prevalence of RF was the only variable significantly associated with positive status to anti-CCP antibodies (P = 0.043). The clinical and biological features of the 12 anti-CCP-positive patients with idiopathic inflammatory myopathy are summarized in Table 1.

**Discussion**

In this cohort of 90 patients with idiopathic inflammatory myopathy, none of whom fulfilled the ACR classification criteria for RA, 13.3 and 0% of serum samples were positive for anti-CCP.
and AKA, respectively. The reported prevalence of anti-CCP was 79% in patients with RA, using the same second-generation anti-CCP assay [16]. To our knowledge, this is the first study to analyse the prevalence of anti-CCP and AKA in a large cohort of patients with idiopathic inflammatory myopathy.

Interestingly, the mean anti-CCP serum titre was high in five patients, moderate in three patients and low in only four patients. Three out of the 12 positive sera (one-third of the patients) showed reactivity >100 U, a threshold that markedly reduces the risk of false-positive results when using a second-generation assay. The mean age of patients and mean disease duration were similar between patients with and without anti-CCP. Hypergammaglobulinaemia could not account for the positive anti-CCP test results, since the mean serum IgG did not vary significantly according to anti-CCP status. Our study confirms that anti-CCP antibodies may be detected in myositis patients with no radiographic evidence of erosive arthropathy after a lengthy follow-up.

The possibility that patients with anti-CCP antibodies might ultimately develop RA cannot be ruled out. It is known that anti-CCP can be present years before the first signs of RA [17]. Additionally, the concomitant presence of RF IgM and anti-CCP, which was observed in five of our patients, is considered the best predictor of the development of active RA [18]. Thus, a cautious clinical and radiographic follow-up is required to confirm the absence of evolution to RA in anti-CCP-positive patients. Nevertheless, the fact that RA did not develop in our cohort of myositis patients over the long-term follow-up makes this possibility unlikely. This study suggests that production of anti-CCP antibodies, which has also been reported in patients with juvenile idiopathic arthritis [19], MCTD [8], SLE [8, 20], SSc [8], autoimmune hepatitis [21], SS [22], PsA [23] and infectious disease (active tuberculosis) [24], may be less intimately related to the pathogenesis of RA than was previously hypothesized.

The fact that none of the patients studied was positive to AKA deserves an explanation. It is possible that antibody specificity in myositis patients is caused by anti-arginine control peptide antibodies rather than citrullinated arginine, as has been demonstrated recently in patients with autoimmune hepatitis and lupus [25]. In this case, the presence of anti-CCP should be interpreted as a false-positive result, although the presence of citrullinated proteins in myositis-affected muscle [26] could suggest that inflammation may play a role in the development of these autoantibodies. Testing for citrulline-specific reactivity with citrullinated and non-citrullinated (arginine) antigen is the only way to ascertain the true reactivity of this antibody. The fact that this issue was not resolved is a limitation of our study. In addition, it should be mentioned that the small number of anti-CCP-positive patients may be the reason why no differences were found between the positive and negative groups.

We suggest that a positive CCP test should be interpreted with caution when contemplating a diagnosis of RA in these patients. Although the patient may present arthritis (even with erosions), this symptom can also be a manifestation of myositis, particularly when anti-synthetase antibodies are present [27]. Nonetheless, a statistically significant association between the presence of anti-CCP and positive status to anti-synthetase antibodies or arthritis was not found in our study.

In conclusion, our data show that most patients with idiopathic inflammatory myopathy are negative for anti-CCP. However, clinicians should be aware that anti-CCP can be present in a small percentage of patients diagnosed with inflammatory myopathy who do not meet the ACR criteria for RA. The negative AKA status of our patients argues against a diagnosis of RA. The fact of anti-CCP positivity did not seem to have particular clinical significance in the cohort of myositis patients studied.

### Acknowledgment

Funding: This study was funded in part by a grant (FIS/2008 PI 08-0450) from Ministerio de Sanidad y Consumo (Spanish Ministry of Health and Consumer Affairs).

Disclosure statement: The authors have declared no conflicts of interest.

### References


