EXTENDED REPORT

Anti-cyclic citrullinated peptide positivity in non-rheumatoid arthritis disease samples: citrulline-dependent or not?

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Background: Antibodies directed against citrullinated proteins (eg anti-cyclic citrullinated peptide (CCP)) have excellent diagnostic and good prognostic potential for rheumatoid arthritis. Type 1 autoimmune hepatitis (AIH-1) is a chronic liver disease characterised by a variety of serum autoantibodies. Recently, in a large group of patients with AIH-1 without clear rheumatoid arthritis overlap, a relatively high percentage (9%) of anti-CCP2 positivity was scored.

Objectives: To characterise the citrulline-dependence of the observed anti-CCP2 positivity in AIH-1 sera as well as in other groups of patients without rheumatoid arthritis (mainly rheumatic diseases).

Methods: Serum samples of 57 patients with AIH-1 and 66 patients without rheumatoid arthritis, most of them reported as anti-CCP positive, were tested for citrulline-specific reactivity with a second generation anti-CCP kit, with the citrullinated and the corresponding non-citrullinated (arginine-containing) antigen. A subset of AIH-1 sera was also tested with a CCP1 ELISA (and arginine control).

Results: The anti-CCP2 reactivity of most non-rheumatoid arthritis rheumatic diseases samples (87–93%) was citrulline-specific, whereas a relatively high percentage of AIH-1 samples (42–50%) turned out to be reactive in a citrulline-independent manner. The use of citrullinated and non-citrullinated CCP1 peptides confirmed a high occurrence of citrulline-independent reactivity in AIH-1 samples.

Conclusions: In rheumatoid arthritis and most non-rheumatoid arthritis rheumatologic disease sera, anti-CCP positivity is citrulline-dependent. However in some patients, particularly patients with AIH-1, citrulline-independent reactivity in the anti-CCP2 test can occur. A positive CCP test in a non-rheumatic disease (eg liver disease) should therefore be interpreted with care, and preferably followed by a control ELISA with a non-citrullinated antigen.

Anti-cyclic citrullinated peptide (anti-CCP) antibodies, belonging to the group of antibodies directed to citrullinated (auto) antigens, are mostly considered as a disease marker for rheumatoid arthritis.^{1–2} Citrulline is a non-standard amino acid, which is generated in proteins by post-translational deimination of arginine residues by peptidylarginine deiminase enzymes.³ Anti-CCP antibodies show both a good sensitivity (77% using a second generation test: CCP2) and a very high specificity for rheumatoid arthritis (99% compared with healthy controls and 95% when compared with patients without rheumatoid arthritis).⁴ They are able to predict the development of rheumatoid arthritis in healthy subjects,⁵ and are prognostic markers of erosive disease progression.⁶ It has also been shown that this marker system can help in discriminating between rheumatoid arthritis and other forms of erosive arthritis that can simulate rheumatoid arthritis.⁷ Anti-CCP antibodies are rarely detectable in other diseases, and in these cases usually with low titres.⁸ Anti-CCP positive rheumatoid arthritis sera 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 recognised by anti-CCP positive rheumatoid arthritis sera.⁹

Autoimmune hepatitis (AIH) is a chronic liver disease of unknown aetiology, characterised by HLA-association, hypergammaglobulinaemia, serum autoantibodies (both liver-specific and non-organ specific) and presence of a dense mononuclear cell infiltrate in the portal tract. Diagnosis of AIH is performed by a cumulative score, which includes clinical, laboratory and histological features, proposed by the International Autoimmune Hepatitis Group (IAHG).^{10–11} Based

on serological and clinical findings, two types of AIH are recognised: in type 1 AIH (AIH-1) antinuclear antibodies (ANA) and/or anti-smooth muscle antibodies (SMA) are detectable, whereas in type 2 AIH (AIH-2) the presence of liver-kidney microsomal antibodies is typical. In both types of disease, women are more often affected than men.¹² Patients with AIH commonly show a good response to immunosuppressive treatment.¹³ AIH may be accompanied by rheumatological manifestations, including arthralgia, symmetrical non-erosive polyarthritis and myalgia.¹⁴ Association between AIH and rheumatoid arthritis is also observed.¹⁵ It has recently been reported that anti-CCP antibodies (using a CCP2 test) can be detected in 9% of patients with AIH-1, in absence of recognisable rheumatoid arthritis overlap, and in some cases with high titres, comparable to those observed in rheumatoid arthritis.¹⁶

The aim of our study was to characterise the observed anti-CCP reactivities in AIH-1, especially regarding their dependence on the citrulline moiety as is the case in rheumatoid arthritis. For this purpose, we tested AIH-1 sera on both CCP citrullinated peptides as well as on the corresponding arginine controls. In parallel, we investigated the dependence of several other diseases on the citrulline moiety, different from rheumatoid arthritis, in which anti-CCP positivity has been described, such as psoriatic arthritis (PsA),^{17–18} palindromic rheumatism¹⁹

Abbreviations: AIH-1, Type 1 autoimmune hepatitis; AIH, autoimmune hepatitis; ANA, antinuclear antibodies; Anti-CCP, anti-cyclic citrullinated peptide; IAHG, International Autoimmune Hepatitis Group; PsA, psoriatic arthritis; SjS, Sjögren's syndrome; SLE, systemic lupus erythematosus; SMA, smooth muscle antibodies

and other rheumatologic conditions (systemic sclerosis, Sjögren's syndrome (SjS), systemic lupus erythematosus (SLE), seronegative arthritis and osteoarthritis).⁷⁻²⁰⁻²³ In these diseases, anti-CCP positivity (for CCP1 or CCP2, with different commercial kits) has been reported with prevalences similar to AIH-1 (PsA, systemic sclerosis, SjS, SLE) or higher (palindromic rheumatism).

MATERIALS AND METHODS

Patient samples

Serum samples were obtained from patients with AIH-1 attending the Department of Internal Medicine, Cardioangiopathy, Hepatology, Division of Internal Medicine, University of Bologna, Bologna, Italy (n = 19; 9 anti-CCP2 positive, previously tested with DIASTAT anti-CCP (Axis-Shield, Dundee, Scotland) performed in accordance with manufacturer's instructions with the recommended 5 U/ml cut-off), from the Department of Medicine, Division of Gastroenterology and Hepatology, University Medical Centre St Radboud, Nijmegen, The Netherlands (n = 12, never tested for anti-CCP, randomly selected), and from the Department of Immunology, Gastroenterology and Hepatology, Erasmus MC, Rotterdam, The Netherlands (n = 26, all anti-CCP2 positive or border-line, as previously tested with ELIA-CCP (PharmaciaDiagnostics, Freiburg, Germany) and selected from a total number of 100 patients with AIH-1). All these 57 patients (12 men (21%) and 45 women (79%)) matched the diagnostic IAHG criteria for AIH-1.¹⁰ Their mean age was 45 (range 7–83) years. ANA were detectable in 30 patients; SMA were detectable in 31 patients; 14 patients had both ANA and SMA; 10 patients were negative for ANA and SMA, but they were retained in the analysis because of their diagnostic score for AIH. Rheumatoid factor was positive in 17 (30%) cases, negative in 35 (61%), dubious in 5 (9%). As for rheumatologic features, nine of them had joint complaints (arthralgias or arthritis), one had overlapping palindromic rheumatism and one had overlapping SjS.

Serum samples from 66 patients with various rheumatologic (non-rheumatoid arthritis) and non-rheumatologic conditions were obtained through kind gifts of different research groups and (where not otherwise stated) from our own collections: 28 with PsA (Dr S Rantapää-Dahlqvist, Umeå, Sweden; n = 11; Dr B Vander Cruyssen and Dr F de Keyser, Ghent, Belgium; n = 15), 7 with SjS (Dr C van Noord, Rotterdam, The Netherlands; n = 2; Dr J-M Anaya, Medellín, Colombia; n = 4), 9 with palindromic rheumatism (Dr R Sanmarti, Barcelona, Spain; n = 9), 13 with SLE (Dr I Hoffman and Dr F De Keyser, Ghent, Belgium; n = 9; Dr J-M Anaya, Medellín, Colombia; n = 3), 3 with systemic sclerosis (Dr J-M Anaya, Medellín, Colombia; n = 1), 2 with erosive osteoarthritis, 1 with seronegative arthritis, 2 with primary biliary cirrhosis and 1 with HCV-related hepatitis. None of these patients had recognisable rheumatoid arthritis overlap. In total, this group consisted of 20 (30%) men and 46 (70%) women. The mean age was 51 (range 18–83) years. In all, 41 (62%) patients were rheumatoid factor positive; 21 (32%) patients were rheumatoid factor negative; status of rheumatoid factor was not available in 4 (6%) cases. All the patients of this group were reported as positive for anti-CCP, previously tested with first or second generation ELISA tests.

Control rheumatoid arthritis sera (n = 41), fulfilling the American College of Rheumatology diagnostic criteria for rheumatoid arthritis and all CCP2 positive, were collected from the Department of Rheumatology of the University Medical Centre St Radboud, Nijmegen, The Netherlands. Control normal sera were obtained from healthy volunteers.

ELISA

Anti-CCP2 ELISA was performed by IMMUNOSCAN RA (Euro-Diagnostica, Arnhem, The Netherlands) in accordance with manufacturer's instructions with the recommended 25 U/ml cut-off. The arginine version of CCP2, in which citrulline in the peptides is replaced by arginine, was tested with a CCP2 control plate (kindly prepared by Euro-Diagnostica, Arnhem, The Netherlands), in accordance with manufacturer's instructions. The cut-off value for the arginine-control plate was arbitrarily determined by the absorbance corresponding to the cut-off (25 U/ml) in the calibration curve for the citrulline-variant. Results are therefore shown as cut-off index (COI; observed OD₄₅₀ over cut-off OD₄₅₀). In house CCP1 ELISA was performed using either streptavidin-coated plates or DNA-bound ELISA plates (Costar, Cambridge, Massachusetts, USA) essentially as described previously.⁹ CCP1 peptides (cit: SHQESTX-GRSRGRSGRSGS; arg: SHQESTRGRSGRSGRSGS) were generously supplied by Dr JW Drijfhout from the Department of Immunohaematology and Blood Transfusion, Leiden University Medical Centre, Leiden, The Netherlands.

RESULTS

Recently, Fusconi *et al*¹⁶ reported anti-CCP positivity in a relatively high percentage of patients with AIH-1. They performed an anti-CCP test (second generation; DIASTAT anti-CCP, Axis-Shield, Dundee, UK) on these patients to investigate whether anti-CCP autoantibodies are part of the large pool of autoantibodies that are present in sera from patients with AIH-1. In their cohort of 133 patients with AIH-1, they observed anti-CCP positivity in 9% (n = 12) of the patients, of which 58% had titres ≥ 20 U/ml (cut-off = 5 U/ml). Of the control rheumatoid arthritis sera, 60% (53 out of 89) were positive. Very low percentages of positivity were observed in primary biliary cirrhosis (2 out of 49) and hepatitis C virus-related disease (1 out of 80) patients, in both cases with titres < 20 U/ml. The anti-CCP test can be performed on patients with chronic hepatitis with arthritic involvement to help in diagnosing a possible coexisting rheumatoid arthritis, for which the test has a reported specificity of 95%.⁴ Given the absolute requirement for the citrulline moiety of the epitope⁸ and the empirically established rheumatoid arthritis-specificity, the diagnostic anti-CCP kits have been optimised for low background of normal sera and only include citrulline-containing antigen(s).

To check whether the reactivity of the anti-CCP positive AIH-1 sera was indeed citrulline-dependent, we tested both CCP2 (IMMUNOSCAN RA kit) and its arginine-control variant (plate supplied by Euro-Diagnostica BV) and compared the results. In parallel, we also tested anti-CCP positive sera from patients with other rheumatologic (non-rheumatoid) and hepatologic conditions. Sera were considered CCP2 positive in accordance with manufacturer's instructions and the recommended 25 U/ml cut-off. The CCP2 positive sera were divided into three groups: (a) citrulline-preferred reactivity when, regardless of their reactivity with the arginine-variant (positive or not), the cut-off index (COI) for the citrulline-variant was > 1 and more than twice the COI for arginine; (b) completely citrulline-specific when they reacted with CCP2, but not with its arginine-variant (this group is obviously a subset of the first group); (c) non-citrulline-specific (citrulline-independent positivity) when the sera reacted with the arginine-variant and the COI for citrulline was less than twice the COI for arginine. Table 1 shows the results for CCP2. In total, we found 12 anti-CCP2 positive patients with AIH-1, with the IMMUNOSCAN RA test (for features of the anti-CCP2 positive patients with AIH-1 see Table 2, where also anti-CCP1 positive cases (see below) are

reported). Out of 12 Dutch patients with AIH-1, never tested for CCP2, 3 (25%) were positive. Regarding the previously tested samples there was some discrepancy: 6 out of 9 DIASAT positive Italian AIH-1 sera were IMMUNOSCAN RA positive positive, whereas only 3 out of 26 EliA positive or borderline AIH-1 sera scored positive in IMMUNOSCAN ELISA. None of the 10 previously reported anti-CCP negative patients with AIH-1 that were tested, reacted with CCP2 by IMMUNOSCAN RA. The CCP2 values measured in AIH-1 sera were in most cases fairly low, which could explain some of the discrepancies between the various tests.

With regard to citrulline-dependency of AIH-1 sera, out of 12 anti-CCP2 positive patients, 7 showed a citrulline-preferred reactivity (6 (50%) of them were completely citrulline-specific) whereas 5 (42%) showed a citrulline-independent positivity. Interestingly, 2 out of the 6 citrulline-specific sera were from patients with an overlapping rheumatic disease (table 2). If we exclude these two cases, the percentage of non-citrulline-specific positivity in patients with AIH-1 is 50% (5 out of 10 patients). Of the three cases with other liver diseases, two reacted as positive with IMMUNOSCAN, and both of them in a citrulline-independent way.

As for the CCP positive rheumatic patients without rheumatoid arthritis, out of 63 tested cases 54 were positive with IMMUNOSCAN CCP2 in our hands; regarding citrulline-dependence, 50 (93%) showed a citrulline-preferred reactivity (47 of them (87%) completely citrulline-specific); only 4 patients (7%) had a citrulline-independent reactivity. Particularly, all the sera from patients with PsA, SjS and palindromic rheumatism displayed a citrulline-preferred reactivity (almost all completely citrulline-specific) (table 1). As for patients with rheumatoid arthritis, all the 41 tested cases reacted with the IMMUNOSCAN kit. As expected, all of them showed a citrulline-preferred reactivity (36 (88%) of them were completely citrulline-dependent) (see table 1 and fig 1). In figure 2 the observed reactivity patterns of individual AIH-1 sera are depicted with connecting lines between the COIs for "citrulline" and "arginine". For the non citrulline-specific sera, most have equal COIs (almost horizontal lines). One serum is far more reactive in the arginine-control ELISA.

Subsets of AIH-1 sera ($n = 31$, 9 anti-CCP2 positive with IMMUNOSCAN and 22 negative) and RA sera ($n = 5$) were also tested for both citrulline- and arginine-variants of the anti-CCP1 test to see if the AIH-1 reactivity was dependent on the antigenic peptides used. The results (shown in Table 2) indicate that the majority of CCP1 reactive AIH-1 sera (12 out of 14 = 86%) did not show a citrulline-preferred reactivity. In

contrast, all the five RA sera used as control showed a citrulline-specific reactivity. Some differences in reactivity for CCP1 compared to CCP2 were observed: all the AIH-1 sera that reacted with CCP2 (IMMUNOSCAN) reacted also with CCP1, except one (case 15, Table 2). Two of these sera changed from citrulline-preferred or citrulline-specific reactivity in CCP2 to citrulline-independent reactivity in CCP1. Six sera that did not react with CCP2 reacted with CCP1, in all cases in a citrulline-independent fashion.

To determine whether components of the ELISA system, independent from the peptides, are responsible for the non-specific reactivity by AIH-1 sera several control experiments (both citrulline- and arginine-variants) in an anti-CCP1 setting were performed (eg, streptavidine-plates instead of DNA-bind plate; with or without bovine serum albumin). Sera maintained their reactivity for CCP1 with these different approaches (results not shown) indicating that the reactivity of the AIH-1 sera was not introduced by the method of testing and that the antibodies really target the presented antigen.

DISCUSSION

Second generation anti-CCP kits contain peptide(s) optimised to avoid reactions that are not specifically directed to the citrulline moiety.²⁴ So far, non citrulline-specific reactivity against citrullinated synthetic peptides has been described only in animal models of arthritis.²⁵ This is the first report regarding human patients, which describes reactivities against synthetic citrullinated peptide(s) which are not specific for the citrulline moiety. This phenomenon definitely occurs in patients with AIH-1: 42% of patients with AIH-1 reacted with CCP2 in a non citrulline-specific manner. This percentage increases to 50% if we do not consider the two patients with an overlapping rheumatic disease (palindromic rheumatism and SjS (table 2)). Next to the AIH-1 sera, the 2 anti-CCP positive patients with other liver diseases (primary biliary cirrhosis and HCV-related hepatitis) also showed a citrulline-independent reaction.

In contrast to these results, in the non-RA rheumatologic patients group, the percentage of citrulline-preferred reactivity is very high (87–93%), and comparable to the patient group with rheumatoid arthritis (88–100%, table 1). Thus, in a rheumatic setting anti-CCP2 positive sera seem to target the citrulline moiety in almost all cases. This is particularly true for PsA, SjS and palindromic rheumatism, in which the rate of citrulline-preferred reactivity is 100%. In these diseases, a positive CCP2 test has to be considered as a genuine presence of antibodies towards the citrulline moiety, also in terms of diagnostic potential for rheumatoid arthritis.

Table 1 Percentages of citrulline-specific reactivity and citrulline-preferred reactivity in sera of different patients groups

Disease	Cases (n)*	CCP2 positive (COI citrulline >1)	Citrulline-preferred (COI citrulline >2xCOI arginine)	Citrulline-specific (COI citrulline >2xCOI arginine; COI arginine <1)	Citrulline-independent (COI arginine >1 and COI citrulline <2xCOI arginine)
AIH-1	57	12	7 (58%)	6 (50%)	5 (42%)
"Pure" AIH-1†	55	10	5 (50%)	4 (40%)	5 (50%)
Psoriatic arthritis	28	27	27 (100%)	27 (100%)	0
Sjögren's syndrome	7	6	6 (100%)	6 (100%)	0
Palindromic rheumatism	9	8	8 (100%)	7 (88%)	0
Systemic lupus erythematosus	13	9	6 (67%)	6 (67%)	3 (33%)
Other rheumatic diseases‡	6	4	3 (75%)	1 (25%)	1 (25%)
Total non-RA rheumatic	63	54	50 (93%)	47 (87%)	4 (7%)
Other liver diseases§	3	2	0	0	2 (100%)
RA	41	41	41 (100%)	36 (88%)	0

AIH-1, Type 1 autoimmune hepatitis; CCP, cyclic citrullinated peptide; COI, cut-off index; RA, rheumatoid arthritis.

*All these cases were previously reported as anti-CCP positive with first or second generation ELISA test, except 10 negative AIH-1 and 12 AIH-1 patients that were not CCP tested before.

†Patients with AIH-1 without overlapping diseases in which anti-CCP production has been reported.

‡Systemic sclerosis ($n = 3$), erosive osteoarthritis ($n = 2$), seronegative arthritis ($n = 1$).

§Primary biliary cirrhosis ($n = 2$), HCV-related hepatitis ($n = 1$).

Table 2 Clinical and laboratory features of anti-CCP2 and/or anti-CCP1 positive patients with AIH-1

Case	Sex	Age	Rheumatologic features	RF	NOS	CCP2 reactivity (U/ml)	CCP1 reactivity
1	M	17	None	Negative	ANA + SMA	<25	Positive *
2	F	16	None	Negative	SMA	<25	Positive *
3	F	72	None	Negative	ANA + SMA	34	Positive *
4	F	23	None	Negative	SMA	41	Positive *
5	F	57	Polyarthritis	Negative	ANA	135	Positive *
6	F	26	None	Negative	SMA	63	Positive *
8	F	35	None	Negative	ANA + SMA	26	Positive *
9	F	20	None	Negative	SMA	<25	Positive *
10	F	16	None	Negative	SMA	509	Positive *
11	F	76	Mild polyarthritis	Negative	ANA	237	Positive †
12	F	65	Palindromic rheumatism	Positive	SMA	332	Positive †
14	F	38	None	Negative	ANA + SMA	<25	Positive *
15	F	69	Sjögren's syndrome; polyarthritis	Positive	ANA	135	Negative —
22	F	38	None	Negative	Neg	<25	Positive *
31	F	49	None	Negative	ANA	<25	Positive *
32	M	73	Polyarthritis	Positive	ANA	81	NT
42	F	32	None	Positive	ANA + SMA	36	NT
55	F	36	None	Positive	ANA	25	NT

ANA, anti-nuclear antibodies; CCP, cyclic citrullinated peptide; F, female; M, male; NOS, non-organ specific antibodies; NT, not tested; RF, rheumatoid factor; SMA, anti-smooth muscle antibodies; —, negative serum.

*Citruiline-independent.

†Completely citruiline-specific.

‡Citruiline-preferred.

Our data show that anti-CCP positive patients with AIH-1 constitute an exception in reactivity towards anti-citrullinated peptides, for their capability to target the peptide moiety rather than the citrulline residue contained in it. This phenomenon is possibly also a common feature in other hepatic diseases, as judged from the few cases we studied. It is at present unclear why in liver diseases, and particularly in AIH-1, this “false” positivity occurs. It is known that in autoimmune hepatitis a wide variety of autoantibodies are produced;²⁶ similarly, in primary biliary cirrhosis and in HCV-related hepatitis various circulating antibodies are detectable.²⁷⁻²⁸ It is also known that in AIH a high level of IgG is common (hypergammaglobulinemia is part of the diagnostic IAHG criteria).¹⁰ However, at least in

AIH-1, a high level of immunoglobulins cannot be the only reason for the reactivity with citrullinated antigens, since no significant difference in immunoglobulins levels between anti-CCP positive and negative AIH-1 patients could be observed.¹⁶ Therefore, the reason of the observed reactivities is probably more related to an extremely broad production of autoantibodies that can target parts of the CCP2 (and other citrullinated peptides) different from the citrulline moiety, rather than the hypergammaglobulinaemia itself. Variations between anti-CCP2 and anti-CCP1 reactivities (table 2) show that the citrulline-independent reactivities are strongly polyclonal responses. Regarding possible differences between the citrulline-dependent and -independent anti-CCP2 positive AIH-1

Figure 1 Reactivity of sera against CCP2 citrulline- and arginine-variant. CCP2 (X) versus arginine-control (R) reactivity of rheumatoid arthritis (RA), autoimmune hepatitis type 1 (AIH-1), psoriatic arthritis (PsA), palindromic rheumatism (PR), systemic lupus erythematosus (SLE), Sjögren's syndrome (SJS), other rheumatologic diseases (ORD) and other hepatic diseases (OHD) sera. Data are depicted as cut-off indices (COIs): the ratio of observed reactivity over the cut-off reactivity (OD value observed at 25 U/ml in citrulline-variant ELISA). The dashed line depicts the COI of 1. Upper limit for the COI was set at 64, representing 1600 U/ml. Each dot represents one serum; mean values are depicted with a horizontal line. The only diseases in which significant reactivity against the arginine-variant is present (mean value >1) are autoimmune hepatitis type 1 (AIH-1), other hepatic diseases (OHD) and a small subset of other rheumatologic diseases (ORD). In OHD the mean value for arginine-control is even higher than the mean value for CCP2.

Figure 2 Corresponding cut-off index (COI) levels for cyclic citrullinated peptide-2 positive individual type 1 autoimmune hepatitis (AIH-1) sera.

sera, the number of cases is too low to allow good statistical analysis. However, it is worth noting that citrulline-dependent positivity seems to be more frequently related to a seropositivity for rheumatoid factor (4 out of 7 citrulline-dependent patients were positive for rheumatoid factor, against 1 out of 5 citrulline-independent). Citrulline-dependent positivity is also more frequently accompanied by rheumatic complaints (4 out of 7 citrulline-dependent sera had rheumatic symptoms or coexisting rheumatic diseases, whereas only 1 out of 5 citrulline-independent patients had rheumatic complaints). These results may indicate that (some of) these patients may have an underlying and not yet recognisable form of early rheumatoid arthritis.

Some discrepancies were observed between the previously reported results and those we obtained with the IMMUNOSCAN RA kit. Most of the previously reported data were obtained with DIASTAT, IMMUNOSCAN and EliA kit. All these kits use the same citrullinated peptide(s) as antigen, but other components of the kits (eg, anti-CCP standards, secondary antibody, cut-off calculation) are different. Discrepancies in results between kits could be due to differences in reagents' composition, especially for determination of low titre positivity (the discrepancies we observed were in fact always occurring in sera with very low titres of reactivity). Possible discrepancies between commercially available second generation anti-CCP ELISA kits have been already shown.²⁹

In conclusion, we showed that some patients without rheumatoid arthritis can produce antibodies directed against the peptide(s) used by the second generation anti-CCP kit, which are not specifically directed to the citrulline moiety. Our data show that this phenomenon occurs especially in liver diseases, particularly AIH-1. Since these autoantibodies are not directed against the citrulline moiety, they must not be considered as genuine anti-CCP antibodies in relation to their diagnostic potential for rheumatoid arthritis. Our data also show that, in a rheumatologic setting, anti-CCP2 autoantibodies are almost always dependent on the presence of the citrulline moiety. Since for some patients without rheumatoid arthritis, in particular patients with AIH-1, citrulline-independent reactivity in the anti-CCP2 test can occur, in such cases

care should be taken in interpreting the results for diagnostic purposes. A control ELISA with a corresponding arginine-containing antigen is then needed to investigate the citrulline-specific recognition.

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