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Short Communication

Comparison of anti-CCP autoantibodies measurement by ELISA and a bead-based assay in a large patient cohort

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ABSTRACT

Objectives: The aims of our study were to compare in a cohort of 705 patients the diagnostic performance of two tests to detect autoantibodies to cyclic citrullinated peptides (CCP) and to determine whether a bead-based assay within a multiplex flow immunoassay (MFA) can be used instead of an enzyme linked immunosorbent assay (ELISA) technique in routine practice.

Design and methods: Six hundred and thirty patients with rheumatic symptoms and 75 patients with systemic lupus erythematosus (SLE) were tested for anti-CCP autoantibodies using two techniques: ELISA (Inova) and MFA (BioPlex®, Bio-Rad).

Results: Using kappa coefficient, there was an excellent agreement between ELISA and MFA when comparing 630 patients with rheumatic symptoms (κ coefficient, 0.82). In this cohort 174 patients were identified as suffering from RA, while 456 patients suffered from other diseases. Sensitivity and specificity values of anti-CCP autoantibodies for RA were 70.7% and 92.3% for ELISA and 64.4% and 92.8% for MFA. The positive and negative predictive values were 77.4% and 89.2% for ELISA and 77.2% and 87.2% for MFA, respectively. There were no differences in the diagnostic performances between the two assays ($Z = 0.67$). The specificity values of anti-CCP autoantibodies analysing patients with SLE were 97.3% with MFA and 96% with ELISA with an excellent agreement between the methods (98.7%; κ coefficient, 0.79).

Conclusion: Concordance between ELISA and MFA is high in routine practice. Overall, MFA is a powerful tool for rapid assessment of anti-CCP autoantibodies and can replace the ELISA technique, which could be used as a second-line test in some cases.

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Introduction

Autoantibodies to cyclic citrullinated peptides (anti-CCP) have demonstrated their usefulness for the diagnosis of rheumatoid arthritis (RA) [1,2]. Recently, the RA classification criteria have included the presence of these autoantibodies as one of the criteria for diagnosis of RA along with clinical and radiological investigations [3]. Since their discovery, several enzyme-linked immunosorbent assays (ELISA) have been commercialized to detect anti-CCP autoantibodies, with the CCP2 and CCP3 tests showing very good performances [4–6]. However, these techniques as currently performed are quite time-consuming and may not provide clinicians with a rapid answer. The aim of our study was to determine whether it is possible in routine practice to use a bead-based assay within a multiplex flow immunoassay (MFA) technique, which is fully automated and more rapid than ELISA. For this purpose, we carried out anti-CCP antibody testing in a large cohort of patients

with rheumatic symptoms whose serum samples were sent to our laboratory for diagnostic investigation or follow-up of RA, and also in systemic lupus erythematosus (SLE) patients in order to assess the specificity of the new MFA test. Indeed, as anti-CCP autoantibodies are biological markers often prescribed to discriminate RA from other rheumatic diseases, a SLE cohort was considered as a relevant control group of autoimmune disease with rheumatic symptoms to study the specificity of such markers for RA.

Materials and methods

Patients

Anti-CCP autoantibodies were measured in serum samples collected from 705 patients. The patients were followed in internal medicine and rheumatology departments. Six hundred and thirty patients (437 women and 193 men, mean age 55 ± 16 years, range 15–92) presented rheumatic symptoms and anti-CCP autoantibodies were analysed as part of diagnostic investigation. Clinical data were obtained. One hundred and seventy-four patients presented newly diagnosed or longstanding RA defined according to the American College of

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Rheumatology criteria [7]. One hundred twenty patients had various autoimmune diseases, 300 had non-autoimmune diseases and 36 unclassified diseases at the time of the study. French observational studies from data obtained from a retrospective study without any additional therapy or monitoring procedure do not need informed consent for the 630 patients as anti-CCP autoantibodies were analysed as part of diagnostic investigation. Seventy-five sera of patients with SLE (66 women and 9 men, mean age 46 ± 14 years, range 17–83) were obtained from a sera collection that was approved by the Institutional Review Board of the Hospices Civils de Lyon (Centre de Ressources Biologiques, Immunobiotech, Hospices Civils de Lyon). Written informed consent was obtained from all SLE patients.

Detection of anti-CCP autoantibodies

Anti-CCP autoantibodies were measured using a bead-based assay within a multiplex flow immunoassay (MFA) (BioPlex™ 2200 anti-CCP, Bio-Rad, Marnes-La-Coquette, France) and ELISA (QUANTA Lite™ CCP3 IgG ELISA, INOVA Instrumentation Laboratory, Paris, France) assays according to the manufacturer's instructions. These two methods use cyclic citrullinated peptides as antigens, with cut-off values of 2.9 kU/L for MFA and 19 kU/L for ELISA. The CCP3 assay was used in this study as this test is performed in our routine practice and has been shown to give the same performance when compared with the CCP2 test on 78 patients (data not shown).

Detection of rheumatoid factor

IgM rheumatoid factor (RF) was measured in the serum of 485 patients from the cohort of 630 patients with rheumatic symptoms, using nephelometry and according to the manufacturer's specifications (BNII® instrument, Siemens Healthcare Diagnostics, Deerfield, IL, USA).

Statistical analysis

Agreement between MFA and ELISA was assessed using the kappa (κ) coefficient (0.01–0.2 indicates slight agreement, 0.21–0.4 fair, 0.41–0.6 moderate, 0.61–0.8 substantial and 0.81–0.99 excellent agreement) [8]. Diagnostic performances were compared by the areas under the receiver operating characteristic (ROC) curves and the 95% confidence interval (CI). Sensitivity, specificity and predictive values were calculated using Microsoft Excel and the statistical software Analyse IT™.

Results

Analytical performance of MFA and ELISA assays

In this study 630 patients with rheumatic symptoms were analysed for anti-CCP autoantibodies measurement by ELISA and MFA. The agreement between the 2 techniques was excellent with a κ coefficient of 0.82 (95% CI 0.77–0.87; SE = 0.027, Z = 30.75, $p < 10^{-9}$). Table 1 summarises the results.

Sensitivity values of anti-CCP autoantibodies for RA were 70.7% using ELISA and 64.4% using MFA with a 2.9 kU/L cut-off point. ROC analysis showed that a cut-off point of 1.9 kU/L for MFA improves sensitivity to 66.9% without important loss of specificity (92.5 vs 92.8%). Of 456 patients with diseases other than RA, 35 were positive with ELISA (specificity 92.3%) and 33 with MFA (specificity 92.8%). The positive and negative predictive values were 77.4% and 89.2% for ELISA and 77.2% and 87.2% for MFA, respectively. There were no differences in the diagnostic performances between the two assays ($z = 0.67$, $p = \text{NS}$). Whatever the technique, the titer of a positive result was not higher in RA patients than in non-RA patients (Fig. 1). The quantitative correlation between the two methods has been performed. Since the two methods use different cut-offs and different linear working ranges, no correlation was observed (data not shown).

Table 1

Prevalence of anti-CCP autoantibodies using two methods in 630 patients undergoing diagnostic investigation.

Method	MFA +	MFA –
ELISA +	131 – 109 RA – 22 non-RA • 8 other AID ^a • 6 non-AID ^b • 8 unclassified	27 – 14 RA – 13 non-RA • 3 other AID ^a • 9 non-AID ^b • 1 unclassified
ELISA –	14 – 3 RA – 11 non-RA • 5 other AID ^a • 5 non-AID ^b • 1 unclassified	458 – 48 RA – 410 non-RA • 102 other AID ^a • 282 non-AID ^b • 26 unclassified

MFA: BioPlex™ 2200 multiplex flow immunoassay (cut-off value of 2.9 kU/L), RA: rheumatoid arthritis; AID: auto-immune disease.

^a Other autoimmune disease (AID): antiphospholipid syndrome, granulomatosis with polyangiitis, Hashimoto thyroiditis, myositis, Sjögren's syndrome, systemic sclerosis, Type 1 diabetes, ulcerative colitis, and systemic lupus erythematosus.

^b Non-autoimmune disease (non-AID): ankylosing spondylitis, arthritis, mastocytosis, osteoarthritis, pulmonary fibrosis, psoriasis, and psoriatic arthritis.

Considering the patients positive for anti-CCP autoantibodies 131 out of 172 patients were positive with both techniques; 109 of those patients had RA, 22 patients presented other diseases: 8 various autoimmune diseases, 6 non-autoimmune diseases and 8 unclassified disease at the time of study. Considering the 458 patients with no anti-CCP autoantibodies, 48 patients had RA, 410 other diseases with 102 various autoimmune diseases, 282 non-autoimmune diseases and 26 unclassified diseases at the time of study.

Discrepant results were observed between the two methods for 41 and 42 patients using MFA cut-off points of 2.9 and 1.9 kU/L, respectively. The clinical data of these patients are presented in Table 1. In this population, the titer of anti-CCP autoantibodies was not higher in RA

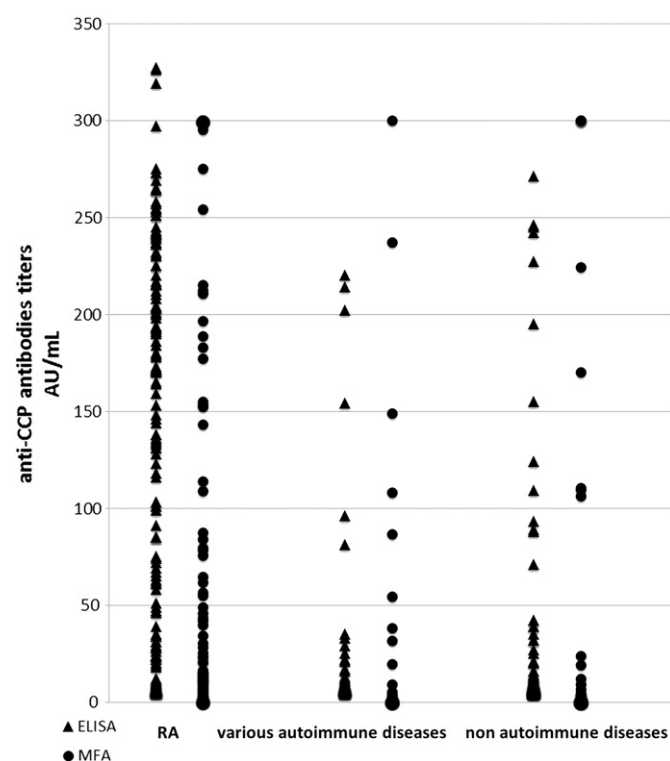


Fig. 1. Titer of anti-CCP autoantibodies (U/mL/kU/L) by ELISA and MFA in a population of 630 patients with rheumatic symptoms. RA: rheumatoid arthritis.

patients than in non-RA patients whatever the technique used (data not shown).

Four hundred eighty five out of 630 patients with rheumatic symptoms were also tested for IgM RF. Comparing with anti-CCP autoantibodies, sensitivity and specificity values of RF were statistically lower ($p < 0.001$) with an AUC of 0.74 (95% CI 0.69–0.78) (Fig. 2).

Specificity of MFA assay

In the cohort of 75 patients with SLE, both methods showed a very good specificity for anti-CCP autoantibodies with 97.3% (2 positive patients) for MFA and 96% (3 positive patients) for ELISA. An overall agreement was confirmed between the two assays of 98.7%, with a calculated κ coefficient of 0.79 showing a substantial agreement (95% CI 0.40–1.19; SE 0.201; $Z = 3.95$, $p < 0.001$).

Advantages of MFA assay

The advantages of the MFA compared with ELISA is that every sample includes an internal standard bead, a serum verification bead and a reagent blank bead to verify detector response, presence of test sample in the reaction vessel and absence of significant non-specific binding, respectively. The time required for the assay is also lower using MFA, with a result obtained in 45 min instead of 120 min by ELISA.

Discussion

Anti-CCP autoantibodies are important serologic support for the diagnosis as well as the prognosis of RA. New techniques like MFA which allows rapid result to the clinicians should be tested on large cohort of patients reflecting everyday rheumatology practice to assess their performance in comparison with current ELISA methods.

This study shows that both ELISA and MFA methods performed equally well to detect anti-CCP autoantibodies, as shown by the similar

diagnostic performances. In our large cohort of 630 patients with RA or rheumatic symptoms possibly related to RA, agreement between the two techniques was excellent with a κ coefficient of 0.82. There was no significant difference in the diagnostic performances between the two tests. Using a MFA cut-off point of 1.9 kU/L improved sensitivity without loss of specificity compared with the 2.9 kU/L cut-off point recommended by the manufacturer. The 1.9 kU/L cut-off point should therefore be considered for use in routine practice. Indeed, most of the cut-offs recommended by the manufacturers should be evaluated by each laboratory in routine practice. The overall sensitivity of 65% for MFA to 71% for ELISA in our study is in agreement with most previously published studies which showed a range of 61.3% to 71.6% [4,5]. Among our patients with anti-CCP autoantibodies, a large percentage (73.2%) of patients had RA while 9.3% had another autoimmune disease such as Sjögren's syndrome, SLE, systemic sclerosis or myositis, and 17.5% had a non-autoimmune disease including ankylosing spondylitis, psoriatic arthritis, psoriasis, osteoarthritis, mastocytosis, pulmonary fibrosis or unclassified disease at the time of study. Our results are thus in accordance with published data on the presence of anti-CCP autoantibodies in some cases of SLE, Sjögren's syndrome, systemic sclerosis and ankylosing spondylitis [9–11]. High titers of anti-CCP autoantibodies were found in some non-RA patients. It is thus difficult to define, on the basis of our study, a cut-off value which can accurately distinguish RA from other rheumatic diseases. Specificity of the marker when tested in a SLE cohort was equivalent between MFA (97.3%) and ELISA (96%). This specificity is in agreement with most previously published studies, which showed a range of 94% to 98.8% [2,10,12]. We asked clinicians to monitor two SLE patients who had a high level of anti-CCP autoantibodies using both techniques, as these biological markers have been shown to be predictors by appearing very early in the course of RA. Thirty-four to 40% of patients with RA in fact had anti-CCP autoantibodies prior to disease onset and 95% of patients with unclassified arthritis and positive anti-CCP autoantibodies developed RA within 3 years [12–17]. The positive and negative predictive values were 77.4% and 89.2% for ELISA and 77.2% and 87.2% for MFA, respectively. These results are in agreement with most previously published studies with an average of 78.4 and 79.8 for positive and negative predictive values respectively [5,6].

As expected, some discrepant results were observed between the techniques since the nature of the cyclic citrullinated peptides used could be different and thus gave different results as already reported for different tests [5]. Regarding the clinical data of the discrepant sera, most patients (60%) had diseases other than RA while 40% of patients had RA.

Comparing with anti-CCP autoantibodies, the specificity of RF was statistically lower ($p < 0.001$) as previously described [18,19]. The lower diagnostic performance for sensitivity can be due to a decrease of RF titer under the cut-off value in longstanding treated RA [20].

In conclusion, the concordance between the two techniques is high enough in routine practice to replace the ELISA technique by MFA, which allows rapid assessment of anti-CCP autoantibodies. However, the ELISA technique could be used as a second-line test in some difficult clinical cases.

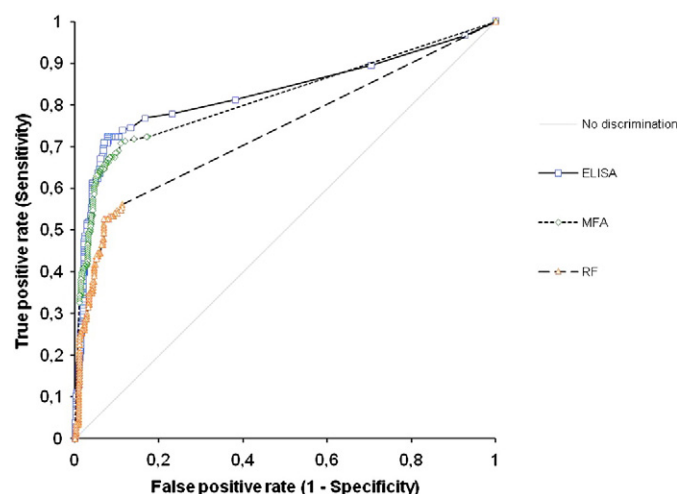


Fig. 2. ROC curves of anti-CCP antibodies by ELISA and MFA as tested on 630 patients with rheumatic symptoms. When standard cut off points were used the diagnostic properties were as follows (95% confidence interval (CI)). ELISA sensitivity 70.7% (95% CI 63.9% to 77.5%), specificity 92.3% (95% CI 90.0% to 94.8%), positive predictive value 77.4% (95% CI 70.9% to 83.9%), and negative predictive value 89.2% (95% CI 86.4% to 92.0%); MFA: Sensitivity 64.4% (95% CI 57.3% to 71.5%), specificity 92.8% (95% CI 90.4% to 95.2%), positive predictive value 77.2% (95% CI 70.4% to 84.0%), and negative predictive value 87.2% (95% CI 84.2% to 90.2%). RF sensitivity 56.2% (95% CI 48.0% to 64.4%), specificity 88.8% (95% CI 85.5% to 92.1%), positive predictive value 67.8% (95% CI 59.4% to 76.2%) and negative predictive value 82.8% (95% CI 78.9% to 86.7%). ROC curve analysis showed areas under curves (AUC) at 0.83 (95% CI: 0.78 to 0.87) for ELISA and 0.82 (95% CI: 0.78 to 0.86) for MFA. There were no differences in the diagnostic performances between the ELISA and MFA assays ($z = 0.67$, $p = \text{NS}$). Comparing the two anti-CCP techniques, diagnostic performances of the RF assay were statistically lower ($p < 0.001$) with an area under the ROC curve at 0.74 (95% IC: 0.69% to 0.78%).

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References

- [1] Lee DM, Schur PH. Clinical utility of the anti-CCP assay in patients with rheumatic diseases. *Ann Rheum Dis* 2003;62:870–4.

- [2] Dubucquoi S, Solau-Gervais E, Lefranc D, Marguerie L, Sibilia J, Goetz J, et al. Evaluation of anti-citrullinated filaggrin antibodies as hallmarks for the diagnosis of rheumatic diseases. *Ann Rheum Dis* 2004;63:415–9.
- [3] Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham III CO, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum* 2010;62:2569–81.
- [4] Mutlu N, Bicakcigil M, Tasan DA, Kaya A, Yavuz S, Ozden AI. Comparative performance analysis of 4 different anti-citrullinated protein assays in the diagnosis of rheumatoid arthritis. *J Rheumatol* 2009;36:491–500.
- [5] Pruijn GJ, Wiik A, van Venrooij WJ. The use of citrullinated peptides and proteins for the diagnosis of rheumatoid arthritis. *Arthritis Res Ther* 2010;12:203.
- [6] Coenen D, Verschueren P, Westhovens R, Bossuyt X. Technical and diagnostic performance of 6 assays for the measurement of citrullinated protein/peptide antibodies in the diagnosis of rheumatoid arthritis. *Clin Chem* 2007;53:498–504.
- [7] Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315–24.
- [8] Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977;33:159–74.
- [9] Gottenberg JE, Mignot S, Nicaise-Rolland P, Cohen-Solal J, Aucouturier F, Goetz J, et al. Prevalence of anti-cyclic citrullinated peptide and anti-keratin antibodies in patients with primary Sjogren's syndrome. *Ann Rheum Dis* 2005;64:114–7.
- [10] Avouac J, Gossec L, Dougados M. Diagnostic and predictive value of anti-cyclic citrullinated protein antibodies in rheumatoid arthritis: a systematic literature review. *Ann Rheum Dis* 2006;65:845–51.
- [11] Fabien N, Goetz J, Sordet C, Humbel RL, Sibilia J. New autoantibodies in rheumatoid arthritis: anti-citrullinated protein or peptide autoantibodies and the others. *Presse Med* 2008;37:1756–66.
- [12] Fabien N, Olsson NO, Goetz J, Johanet C, Escande A, Bardin N, et al. Prevalence of autoantibodies to cyclic citrullinated peptide in patients with rheumatic diseases other than rheumatoid arthritis: a French multicenter study. *Clin Rev Allergy Immunol* 2008;34:40–4.
- [13] Kudo-Tanaka E, Ohshima S, Ishii M, Mima T, Matsushita M, Azuma N, et al. Autoantibodies to cyclic citrullinated peptide 2 (CCP2) are superior to other potential diagnostic biomarkers for predicting rheumatoid arthritis in early undifferentiated arthritis. *Clin Rheumatol* 2007;26:1627–33.
- [14] Combe B, Landewe R, Lukas C, Bolosiu HD, Breedveld F, Dougados M, et al. EULAR recommendations for the management of early arthritis: report of a task force of the European Standing Committee for International Clinical Studies Including Therapeutics (ESCISIT). *Ann Rheum Dis* 2007;66:34–45.
- [15] Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004;50:380–6.
- [16] Raza K, Breese M, Nightingale P, Kumar K, Potter T, Carruthers DM, et al. Predictive value of antibodies to cyclic citrullinated peptide in patients with very early inflammatory arthritis. *J Rheumatol* 2005;32:231–8.
- [17] van Gaalen FA, Linn-Rasker SP, van Venrooij WJ, de Jong BA, Breedveld FC, Verweij CL, et al. Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: a prospective cohort study. *Arthritis Rheum* 2004;50:709–15.
- [18] Vallbracht I, Helmke K. Additional diagnostic and clinical value of anti-cyclic citrullinated peptide antibodies compared with rheumatoid factor isotypes in rheumatoid arthritis. *Autoimmun Rev* 2005;4:389–94.
- [19] Nishimura K, Sugiyama D, Kogata Y, Tsuji G, Nakazawa T, Kawano S, et al. Meta-analysis: diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. *Ann Intern Med* 2007;146:797–808.
- [20] De Rycke L, Verhelst X, Kruithof E, Van den Bosch F, Hoffman IE, Veys EM, et al. Rheumatoid factor, but not anti-cyclic citrullinated peptide antibodies, is modulated by infliximab treatment in rheumatoid arthritis. *Ann Rheum Dis* 2005;64:299–302.