Clinica Chimica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Anti-citrullinated peptide antibodies and rheumatoid factor isotypes in the diagnosis of rheumatoid arthritis: An assessment of combined tests

Maria Infantino [a,*], Mariangela Manfredi [a], Francesca Meacci [a], Piercarlo Sarzi-Puttini [b], Cristian Ricci [c], Fabiola Atzeni [b], Maurizio Benucci [d]

[a] Immunology and Allergology Laboratory Unit, S. Giovanni di Dio Hospital, Florence, Italy
[b] Rheumatology Unit, L. Sacco University Hospital, Milan, Italy
[c] Department of Epidemiology and Preventive Medicine, University of Regensburg, Regensburg, Germany
[d] Rheumatology Unit, S. Giovanni di Dio Hospital, Florence, Italy

ARTICLE INFO

Article history:
Received 2 February 2014
Received in revised form 17 May 2014
Accepted 23 May 2014
Available online xxxx

Keywords:
Rheumatoid factor
Anti-CCP
Rheumatoid arthritis

ABSTRACT

ACPA (anti-citrullinated protein antibody) tests are today systematically added to clinical and radiological investigations when diagnosing rheumatoid arthritis (RA), and the inclusion of ACPA positivity in the new 2010 RA criteria underlines their importance.
The aim of this study was to determine the sensitivity and specificity of different ACPA assays and IgA, IgG and IgM isotypes of rheumatoid factor (RF) in a cohort of patients with early RA in order to assess the value of combining the tests. The serum samples were obtained from 46 RA patients, 80 patients with systemic rheumatic disease, and 20 blood donors. ACPAs were measured using five different commercial kits.
The receiver operating characteristic (ROC) curves of the anti-ACPA tests had area under the curve (AUC) values of 0.60–0.83. The diagnostic accuracy of the Bio-Rad multiplex flow immunoassay, a new technology for ACPA testing, was very similar to that of the other widely used commercial immunoassays. The EliA CCP-Phadia test was the most the most specific, and had the best positive likelihood ratio and positive predictive values, whereas the anti-CCP Inova 3.1 test was the most sensitive, and had the best negative likelihood ratio and negative predictive values.
The best combination to use for early RA screening was an ACPA test together with IgM and IgA RF.

1. Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disease characterised by chronic joint inflammation (which often leads to the destruction of bone and cartilage) and the presence of autoantibodies, including rheumatoid factor (RF) and highly RA-specific anti-citrullinated protein antibodies (ACPAs usually measured as anti-CCP) [1]. It has been shown that RF and ACPA are present before the appearance of the clinical symptoms of arthritis, thus suggesting that the initial immune dysregulation occurs years before symptomatic disease [2]. It has also been shown that ACPAs are specific prognostic markers, and predict the erosive or non-erosive progression of the disease, thus making them useful for the optimal therapeutic management of RA patients [3].

Anti-keratin antibodies (AKAs) directed against fillagrin and anti-perinuclear factor (APF) have been historically described in RA patients, whereas ACPAs bind to citrullinated filaggrin, an epithelial protein containing citrulline residues as a result of post-translational modification [4]. In 1998, Shellekens *et al.* reported that AKAs recognise an epitope of citrullinated peptides in the serum of RA patients, and this led to the development of the first-generation ACPA test (designated anti-CCP), which used a mixture of CCP as a coating. However, its sensitivity was no more than 50%, and it was later replaced by second- and third-generation tests (CCP2 and CCP3), which used a mixture of synthetic cyclic peptides as a coating and increased sensitivity to 80% [5,6]. The currently available ACPA assays use one of two synthetic peptide mixtures: CCP2 (Euro-Diagnostica) or CCP3 (Inova) [7]. The CCP2 peptide sequence was identified by screening highly complex peptide libraries using highly reactive serum taken from RA patients, whereas CCP3 was designed by means of combinatorial peptide engineering and contains multiple citrullinated epitopes in a conformational structure that increases epitope exposure and immunoreactivity, especially in the case of early RA [8]. Because of patent restrictions, most manufactures use the same synthetic peptide mixture as a coating, which means that

* Corresponding author at: Immunology and Allergology Laboratory Unit, Ospedale S. Giovanni di Dio, Via Torregalli 3, 50143 Firenze, Italy. Tel.: +39 0556932016; fax: +39 0556932289.
E-mail address: maria2.infantino@asf.toscana.it (M. Infantino).

http://dx.doi.org/10.1016/j.cca.2014.05.019
0009-8981/

the differences between them are more due to the method/instrument than the coating itself.

Today, ACPA tests are systematically added to clinical and radiological investigations when diagnosing RA, and the inclusion of ACPA positivity in the new 2010 RA criteria underlines their importance: RF and ACPAs together have a positive predictive value of nearly 100% [9].

The first international reference ACPA preparation has now been developed, an important step in reducing inter-laboratory and inter-method variability [10]. Furthermore, increasing demand has led a number of manufacturers to improve their own methods, which are mainly based on enzyme-linked immunosorbent assays (ELISAs), although a new multiplex flow immunoassay has recently been set up and shown very good concordance with ELISA [11].

The primary aim of this study was to determine the sensitivity and specificity of different ACPA assays and IgA, IgG and IgM isotypes of RF in a cohort of patients with early RA in order to assess the value of combining the tests [12–14]. The secondary aim was to compare the diagnostic sensitivity and specificity of the newly available ACPA assays with the previous tests.

2. Material and methods

2.1. Serum sampling

Serum samples were obtained from 46 patients with early RA diagnosed on the basis of the 2010 American College of Rheumatology (ACR) criteria (42 females and four males; mean age 65.2 ± 7.3 years; disease duration 1.5 [0.5–5.2] months). who attended the Rheumatology Unit of San Giovanni di Dio Hospital, Florence (Italy), between July 2011 and April 2012. Three of the patients were being treated with one or more immunosuppressants (methotrexate, sulfasalazine) and 43 with one of the biological drugs available in Italy at that time (infliximab, adalimumab, etenarcept, certolizumab pegol, rituximab, tocilizumab or abatacept) because they were non-responders to DMARD treatment or had experienced adverse events (Table 1). The 28-joint disease activity score (DAS28) was evaluated at baseline and every 3 months.

The controls consisted of 100 samples taken from 22 patients with spondyloarthritis (SpA), 23 with psoriatic arthritis (PsA), 15 with systemic lupus erythematosus (SLE), eight with primary Sjögren's syndrome (SS), 12 with other well-defined autoimmune diseases (systemic sclerosis, polymyositis, etc.), and 20 blood donors (72 females and 28 males; mean age 58 ± 8.2 years).

All of the patients signed an informed consent form in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of San Giovanni di Dio Hospital.

2.2. Serological assays

The assays were performed in the Laboratory of Immunology and Allergology of San Giovanni di Dio Hospital using aliquoted sera stored at −80 °C. ACPAs were measured using the following commercial kits: anti-CCP (Axis-Shield Diagnostics, Dundee, UK), QuantaLite CCP 3.1 (INOVA Diagnostics, Inc., San Diego, CA), QuantaLite CCP 3 (INOVA Diagnostics, Inc., San Diego, CA), EliA CCP (Phadia AB, Uppsala, Sweden), anti-CCP high sensitive (Orgentec Diagnostika GmbH, Mainz, Germany), and anti-CCP (Bio-Rad Laboratories, Hercules, CA). RF IgM was measured using a BNII nephelometric analyser (Siemens, Marburg, Germany), and RF IgG and IgA were measured by means of enzyme immunoassays (Orgentec Diagnostika) (Table 2). The reagent kits came from the same lot in order to avoid lot variability.

The tests were carried out using a DSX instrument (Dynex Technologies GmbH, Denkendorf, Germany) except in the case of the Bio-Rad and Phadia kits, which were respectively used on a BioPlex 2200 and Phadia® 250. All of the assays were performed in accordance with the manufacturers' instructions. The calibrators and controls were run in duplicate, and the samples in single determinations.

The samples with values outside the analytical measuring range were retested in order to confirm the results.

Table 1
Characteristics of the study population.

No. of patients with RA diagnosis	46
Mean age, years	65.2 ± 7.3
Gender (M/F)	6/40
Duration of disease, months (SD)	1.5 ± 0.5–5.2
No. of subjects with non-RA diagnosis	100
Psoriatic arthritis	23
Spondyloarthritis	22
Systemic lupus	15
Sjögren's syndrome	8
Other autoimmune diseases	12
Blood donors	20

2.3. Statistical analysis

The subjects' age and disease duration are described using mean values and standard deviations or median values and ranges, depending on the skewness of the variable. In order to investigate the relationship between anti-CCP concentrations and DAS28 scores, the results of the ACPA assays were dichotomised using a threshold of 200 U/mL and the dichotomised categories were compared with the DAS28 scores using the Mann–Whitney *U* test. The data were analysed using Analyse-it™ statistical software, version 2.24 (Analyse-it Software Ltd., Leeds, UK). The performance parameters (sensitivity, specificity, positive likelihood ratio [LR+], negative likelihood ratio [LR−], positive predictive value [PPV], negative predictive value [NPV], and kappa agreement) were calculated using the manufacturers' cut-off values for each assay, and receiver operator characteristics (ROC) curves were drawn up.

3. Results

At the manufacturers' cut-off range values varied from 45.65% to 73.91% for diagnostic sensitivity and from 87% to 95% for diagnostic specificity.

The EliA CCP-Phadia test was the most specific of the APCA assays (95%), and had the best LR+ and PPV, although the second-best performers (Bio-Rad CCP and Axis-Shield CCP) were also very specific.

The EliA CCP-Phadia already reaches the 95% specificity at manufacturer cut-off, while others' cut-off already needs to be increased.

INOVA 3.1 was the most sensitive test (73.9%) with the best LR− and NPV and at a predefined specificity of 98% it performed the best sensitivity value (45.7%) together with Axis-Shield CCP (Table 3).

The rise in cut-off to a specificity of 98% had the highest impact on EliA CCP-Phadia and BioPlex CCP probably because the assays had the highest number of patient samples in the measuring range between 95%-cut-off and 98%-cut-off and so they had a higher differentiation ability in this high positive range.

Among the RF assays, only IgM and IgM + IgA were specific, but their sensitivity was poor. The many false positive RF IgG results reduced the specificity of the IgM + IgA + IgG combination.

Combining ACPA testing with RF IgM or RF IgM + RF IgA improved sensitivity, but reduced specificity. The RF isotypes combined with EliA CCP-Phadia were always more sensitive than the other ACPA/RF isotype combinations (Table 4).

There was substantial agreement among the methods (80.1%–96.5%) (Table 5).

The ROC performance curves showed area under the curve (AUC) values of 0.68–0.83 for the ACPA tests, and values of 0.68–0.77 for the RF isotypes (Fig. 1).

There was no statistically significant difference in the AUCs of the different assays, except for anti-CCP Orgentec (Table 6).

Please cite this article as: Infantino M, et al, Anti-citrullinated peptide antibodies and rheumatoid factor isotypes in the diagnosis of rheumatoid arthritis: An assessment of combined tests, Clin Chim Acta (2014), http://dx.doi.org/10.1016/j.cca.2014.05.019