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Comparison of Anti-CCP Peptide with Rheumatoid Factor and its Isotypes for Early Differential Diagnosis and Prognosis of Rheumatoid Arthritis

[1]Margaret T. Agyei-Frempong, [2]Samuel A. Sakyi and [3]Robert E. Quansah

The need for a specific standard test for Rheumatoid Arthritis (RA) cannot be overemphasized. In many instances, either the clinical diagnosis or the disease classification according to America College of Rheumatology (ACR) criteria is used as the basis for defining whether the patient has RA or not. However, predicting the clinical outcome of RA in a patient is more important for therapeutic decision making than predicting whether an arthritis syndrome will ever satisfy a set of classification criteria. The aim of this study was to compare anti-Cyclic Citrullinated Peptide test with those of Rheumatoid Factor (RF) and its isotypes RF-IgA and RF-IgM for early and differential diagnosis of RA. In a cross-sectional study, the concentrations of RF and its isotypes RF-IgA and RF-IgM and anti-Cyclic Citrullinated Peptide were determined in 102 classified and unclassified RA patients and 50 healthy controls. Sensitivity was highest for RF-IgM (58.45%), followed by anti-CCP (54.34%), RF latex (52.48%) and RF-IgA (28.51%). Specificity was highest for Anti-CCP (96.67%), followed by RF-IgM (69.52%), RF-Latex (63.12%) and RF-IgA (51.75%). The high specificity of anti-CCP combined with the high sensitivity of RF-IgM infers that a combination of anti-CCP and RF-IgM is a good predictor for early diagnosis and course of RA.

Key words: Rheumatoid arthritis, anti-cyclic citrullinated peptide antibodies, rheumatoid factor., Rheumatoid factor-immunoglobulin M and A (RF-IgM, RF-IgA)

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For further information about this article or if you need reprints, please contact:

Margaret T. Agyei-Frempong
Department of Molecular Medicine,
School of Medical Sciences,
College of Health Sciences,
Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Tel: +233208186136
Fax: +2335160137

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[1]Department of Molecular Medicine, School of Medical Sciences,
Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[2]Department of Clinical Biochemistry, Komfo Anokye Teaching Hospital,
Kumasi, Ghana
[3]Department of Surgery, Trauma Unit, School of Medical Sciences,
Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

INTRODUCTION

The need for a specific standard test for Rheumatoid Arthritis (RA) cannot be overemphasized. In many instances, either the clinical diagnosis or the disease classification according to America College of Rheumatology (ACR) criteria is used as the basis for defining whether the patient has RA or not. However, predicting the clinical outcome of RA in a patient is more important for therapeutic decision making than predicting whether an arthritis syndrome will ever satisfy a set of classification criteria. The American College of Rheumatology (ACR) criteria are based mainly on clinical parameters (Arnett *et al*., 1988). Since, these parameters are often only sufficiently fulfilled when the damaging effects of the inflammatory process are already in progress, this set of criteria is not very suitable for the early diagnosis of RA.

Arthritis is the inflammation of one or more joints. Many different diseases, including gout, Rheumatoid Arthritis (RA), Ankylosing Spondylitis (AS) and osteoarthritis (OA), produce joint pains, however, rheumatoid arthritis is the most serious, painful and potentially crippling form of it (Berglin *et al*., 2006). Rheumatoid Arthritis (RA) is a chronic inflammatory multisystem autoimmune disorder of undetermined aetiology involving primarily the synovial membranes and articular structures of multiple joints (Van Boekel *et al*., 2001). Until recently, treatment for RA was limited and severe joint damage and overall debility were common. Early and aggressive intervention with new and effective biological agents can alter the course of the disease, reverse morbidity and increase life expectancy (De Vries-Bouwstra *et al*., 2005).

Autoantibodies are common characteristic features of rheumatic autoimmune diseases. However, most of the autoantibodies are not disease specific making it extremely difficult to distinguish it from other joint and bone diseases (Nielen *et al*., 2004). Recent studies have however, demonstrated that anti-Cyclic Citrullinated Peptide (CCP) antibodies are highly specific and are sensitive for RA (Kroot *et al*., 2000; Schellenkens *et al*., 1998).

Together with classical clinical features of the disease, serological abnormalities such as the presence of Rheumatoid Factors (RF) and anti-CCP have been shown to be useful diagnostic tools particularly in the early stages of the disease and predictive of disease progression (Meyer *et al*., 2003).

Besides, anti-CCP appears to be a good prognostic marker capable of discriminating between erosive and non-erosive disease. These autoantibodies bind antigenic determinant that contain the unusual amino acid citrulline formed by post-translational modification of arginine residues by peptidylarginine deiminase. The anti-CCP antibody test has subsequently become available commercially and its diagnostic accuracy has been compared with RF test in some countries (Schellenkens *et al*., 2000).

However, it has never been tried in Ghana. This study was therefore set up to compare the sensitivity and specificity of anti-CCP with those of RF and its isotypes RF-IgA and RF-IgM for early and differential diagnosis of RA in Ghana.

MATERIALS AND METHODS

This study was carried out at the School of Medical Sciences of the Kwame Nkrumah University of Science and Technology, Komfo Anokye Teaching Hospital, Kumasi and MEDILAB DIAGNOSTIC centres in Accra, Kumasi, Obuasi, Sunyani and Techiman. This was a prospective study covering a period from October 2006 to June 2008.

Patients: One hundred and sixty seven suspected RA patients, visiting the orthopaedic and chemical pathology units of KATH and the chemical pathology units of MEDILAB DIAGNOSTIC centres in Accra, Kumasi, Obuasi, Sunyani and Techiman and who consented to participate in the study, were administered with standardized questionnaires. The criteria for patient's selection also included: 18 years and older either suspected to have, or diagnosed with arthritis and /or chronic joint pains.

Fifty healthy blood donors from KATH blood donor clinic, who had not been diagnosed of arthritis or chronic joint pains or any inflammatory condition and who consented to the study, were used as controls.

Sample collection: Six milliliters of venous blood were collected from each of the 167 patients who consented to the study as well as the 50 controls. Two milliliters of the blood were placed in EDTA tubes for haematological studies using Sysmex XT-2000i. The ESR was determined using the Westerngren tube method, one part of disodium citrate to four part of whole blood (Expert Panel on Blood Rheology of the International Council for Standardization for Haematology, 1993).

The remaining 4 mL were processed and stored at -70°C until assayed and were brought to room temperature before assay. Anti-Cyclic citrullinated peptide (anti-CCP) antibodies were determined quantitatively by Enzyme-Linked Immunosorbent Assay (ELISA) technique using AXIS-SHIELD DIASTAT reagent kits and ELISA reader

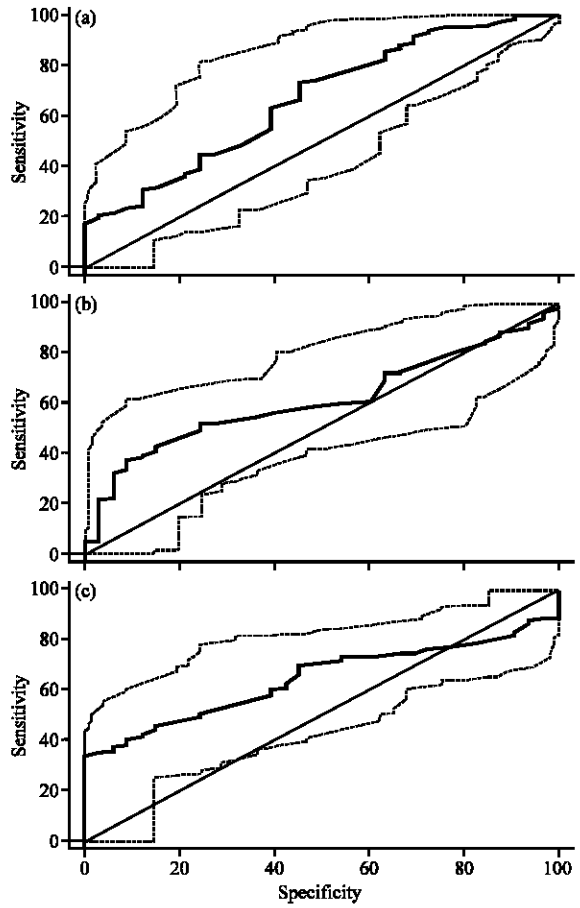

Fig. 1: The graphical representation of sensitivities and specificities for (a) RF IgA, (b) RF-IgM and (c) Anti-CCP

(DNM 9602 New Tech Corp.) and ELISA washer (DNX9620 New Tech Corp).

Rheumatoid Factor (RF) was determined both qualitatively and semi-quantitatively by latex agglutination slide test using reagents from the Smartest Diagnostics (USA). The Diagnostic Automation, Inc. (DAI) Rheumatoid Factors RF-IgM ELISA was used for the detection of IgM antibodies to RF antigens in patients' sera.

Autostat II RF IgA ELISA was used for the quantitative determination of IgA in patients' sera. Figure 1a-c shows the graphical representation of sensitivities and specificities for RF IgA, RF-IgM and Anti-CCP, respectively.

Statistical analysis: All data analysis in this research was done using GraphPad Prism for Windows version 4.02 (GraphPad Software, San Diego, CA, USA) and MEDCALC (version 9.4.2.0). The results were given as Mean±Standard Error of Mean (SEM). The Receiver Operator Characteristic (ROC) was used to analyze the sensitivity and specificity of the samples. For all statistical comparisons, the level of significance was set at $p<0.05$. The cut-off value for the positivity of the anti-CCP antibody, RF-IgA and RF-IgM test were determined from the Receiver Operating Characteristic (ROC) curve and the area under the ROC curve computed.

Table 1: The sensitivities and specificities of the various tests and their statistical significance in the detection of RA

Assay	Sensitivity 95% CI	Specificity 95% CI
Anti-CCP antibodies	0.54 (0.50-0.75)	0.96 (0.86-1.00)
IgM-RF	0.58 (0.50-0.65)	0.69 (0.60-0.98)
IgA-RF	0.28 (0.30-0.65)	0.51 (0.60-0.90)
RF-latex	0.52 (0.48-0.65)	0.63 (0.60-0.90)

Table 2: Comparison of agreement in identifying RA patient as positives and controls as negative (Kappa's test)

Parameters	Agreement among RA patient	Agreement among control patient
Anti-CCP vs. IgM-RF	0.75 (0.44-0.78)	0.03 (-0.30-0.00)
Anti-CCP vs. IgA-RF	0.50 (0.20-0.40)	0.07 (-0.10-0.50)
Anti-CCP vs. RF-latex	0.55 (0.20-0.45)	0.05 (-0.20-0.30)

The sensitivities (among RA seronegative patients) and specificities (among RA seropositive patients) were computed for each of the four tests, along with the 95% Confidence Intervals (CI) as shown in Table 1.

A measure of agreement to examine whether the tests tended to identify the same patients as positive or negative was also computed. The kappa statistic measures agreement beyond chance; a value of 0 implies no agreement beyond chance and 1 implies perfect agreement. Values less than 0.5 were interpreted as representing poor agreement, 0.5-0.75 fair agreement and >0.75 excellent agreement as indicated in Table 2.

RESULTS

Descriptive data: Out of the 167 suspected RA patients, 102 patients fulfilled four or more conditions of revised edition of America College of Rheumatology (ACR) criteria for RA. Out of the 102, 68 were females and 34 were males with ratio of female: male as 2:1. Median age was 49.96 years for females and 46.75 years for males. Heamatological inference on all the patients examined denoted a normocytic, normochromic anaemia with a mean heamoglobin of 11.3 g dL^{-1}. ESR ranged from 2-87 mm h^{-1} and 8-88 mm h^{-1} for males and females respectively, a total mean of 34.63 mm h^{-1} and a standard deviation of 24.17 as shown in Table 3. The mean of anti-CCP, RF-Latex, RF-IgA and RF-IgM for patients and controls are shown in Table 4.

Sensitivity and specificity of anti-CCP antibodies, RF-Latex, IgM-RF and IgA-RF: Sensitivity for RA was highest for RF-IgM test (58.45%) followed by anti-CCP

Table 3: Mean characteristic features of RF-IgA, RF-IgM, RF-Latex and anti-CCP antibodies for patients presenting with arthritis

Characteristics	Values
Mean age	49 years (22-73)
Mean symptom duration	>8 months (SD 5.9)
Female: male ratio	2:1
RF-IgA-positive	27.4%
RF-Latex-positive	41.2%
Anti-CCP-positive	72.5% (titre >5 pg mL^{-1})
RF-IgM-positive	52.9%
Mean ESR	34.6 mm h^{-1}

Table 4: General characteristics of the subjects and control

Parameters	Test	p-value	Control
Age (years)	49.12±0.89	0.0002	48.16±1.34
HB (g dL^{-1})	11.27±0.10	0.0001	13.99±0.15
ESR (mm h^{-1})	34.64±1.87	0.0001	9.23±0.91
RF IgM (IU L^{-1})	1.79±0.04	0.0001	0.55±0.04
RF IgA (IU L^{-1})	17.62±0.29	0.0002	15.51±0.37
Anti-CCP (pg mL^{-1})	5.73±0.15	0.0001	2.13±0.08
RF-Latex (IU L^{-1})	2.34±0.54	0.0002	1.87±0.05

The values are expressed as Mean±SEM

Table 5: Demographic characteristics and biological data for patients resenting with arthritis

Age group	n	%	No. of ACR criteria	Symptom duration (months)	Mean Anti-CCP value
21-30	2	1.2	3	<1	5.10
31-40	27	16.2	4	1-6	5.45
41-50	49	29.3	5	7-9	5.80
51-60	51	30.5	5	10-12	5.82
61-70	26	15.6	6	13	6.10
>70	12	7.2	6	>13	7.44

Distributions are described as n (%) per category and median (range) for qualitative and quantitative items, respectively. Anti-CCP value expressed in pg mL^{-1}

antibody (54.34%), RF-latex (52.48%) and RF-IgA tests (28.51%). Specificity of 96.67% was obtained for anti-CCP, 69.52% for RF-IgM, 63.12% for RF-Latex and 51.75% for RF-IgA. The agreement between these tests in their ability to detect RA patients as positive was low ($\kappa<0.4$).

There was no agreement beyond what could be expected by chance between anti-CCP, IgM-RF and RF-latex in their tendency to generate false-positive results among the controls. Differences in test sensitivity between men and women and across age groups were not statistically significant. There were no differences in test sensitivity according to disease duration or number of ACR RA criteria other than RF positivity fulfilled as shown in Table 5.

DISCUSSION

In many early cases of RA, clinical symptoms are milder and nonspecific and patients will not fulfil all ACR classification criteria for RA. Therefore, the detection of a disease-specific autoantibody like anti-CCP is of great diagnostic and therapeutic importance. In this study anti-CCP antibodies were detected in 52% of patients with less than six months duration. This is comparable to that of Nell *et al.* (2004) in which anti-CCP antibodies were detected in roughly 50-60% of patients with early RA at baseline (usually less than 3 months of symptoms), as indicated in Table 5.

Most of the studies that have compared anti-CCP and RF isotypes assessed RF-IgM alone as a single marker (Larkin *et al.*, 1986). In the present study, anti-CCP was compared not only with RF-IgM but also with RF-Latex and RF-IgA in a population which fulfilled four or more of ACR criteria as well as those with less than four symptoms who therefore do not fulfil ACR criteria.

The specificity obtained for the anti-CCP test (96.6%) in this study is similar to those obtained by Schellekens *et al.* (2000) (97.8%), Mansky *et al.* (2000) (91.8%) and Bizzaro *et al.* (2001) (95.4%). The specificity of the anti-CCP antibody test (96.6%) was significantly higher ($p<0.001$) than that for RF-IgM (69.5%) and the other RF isotypes.

The sensitivity for anti-CCP (54.34%) however, did not significantly differ from that of IgM (58%) and RF (52%) respectively. Although there is some consensus from various studies in the literature about specificity, there are variations in diagnostic sensitivity ranging from 40-75% (Bas *et al.*, 2003).These variations can be due to different cut-off values and differences in serum dilutions, unit of expression or determined from ROC curve. The sensitivities obtained for RF-Latex, RF-IgM and RF-IgA were similar to that of anti-CCP antibody, hence the better diagnostic accuracy of anti-CCP antibody was mainly due to its higher specificity. The anti-CCP antibody test has moderate sensitivity and excellent specificity, RF-IgA has poor sensitivity and moderate specificity. RF-IgM and RF-Latex have moderate sensitivities and good specificities, thus, while negative results for RF-IgA and RF-IgM do not rule out the diagnosis of RA, positive tests for anti-CCP antibodies and RF-IgM practically establish diagnosis. The combination of anti-CCP antibody and IgM-RF positivity improved specificity over RF positivity alone.

Nevertheless, Dahlqvist *et al.* (2003), demonstrated that anti-CCP and IgA-RF predict the development of rheumatoid arthritis in pre-disease serum samples. The mean anti-CCP (5.73±0.15) in the patients was significantly higher ($p<0.0001$) than that of the control (3.13±0.08).

The specificity of anti-CCP extends to patients with early disease in whom a timely diagnosis is most needed, for example, those with symptoms of duration from 1-6 months have a mean anti-CCP of 5.45 pg mL^{-1} thus anti-CCP antibodies also identify subsets of patients who are likely to have substantial ongoing disease activity, accrue more damage and will benefit from early aggressive treatment. The low sensitivity of the test

(40-75%) in most published cohort (Bas *et al*., 2003; Neuhauser *et al*., 1999) indicates that a negative anti-CCP antibody does not exclude the disease, but its high specificity means that a positive result increases the probability that the patient will have RA.

This study also demonstrated the additional prognostic value of anti-CCP antibodies in patients with severe joint destruction and active disease compared with the different RF isotypes; for example anti-CCP was detected in up to 24.4% of IgM-RF negative sera and 30.5% of combined RF-IgM and RF-IgA negative sera of suspected rheumatoid patients who satisfied four or more of ACR criteria, this is similar to that obtained by Vallbracht *et al*. (2004).

The presence of anti-CCP antibodies has already been proposed for inclusion among the classification/ diagnostic criteria for RA (Wiik *et al*., 2004). As a screening method for rheumatoid arthritis, the IgM-RF and the anti-CCP assays are superior to other RF isotypes. This set of diagnostic and prognostic markers would allow the clinician to choose a more powerful disease modifying antirheumatic drug early in the course of disease, even when clinical judgment might not yet indicate the need for such drugs.

CONCLUSIONS

This study has demonstrated that the combination of anti-CCP and RF-IgM assay is highly specific and moderately sensitive for RA, making this combination of autoantibodies a powerful serologic tool in the serologic assessment of RA in Ghana. Anti-CCP alone is also highly specific and moderately sensitive for RA. A positive anti-CCP result in seronegative RA strongly supports the diagnosis of RA serologically.

The presence of anti-CCP at disease onset means that they have a high positive predictive value for the development of erosive joint lesions and the detection of these antibodies can therefore be used in clinical practice to help plan a therapeutic strategy. In view of the high specificity of these antibodies, the test is particularly useful in differential diagnosis between RA and other arthritides that are clinically similar to RA and may be positive for Rheumatoid Factor (RF). Overall, the discriminative ability of the anti-CCP test is impressive; if a patient with RA and control were to be selected at random, the odds are that the RA patient would have the higher anti-CCP antibody concentration.

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